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(54) Title: VACCINE FOR PERIODONTAL DISEASE

(57) Abstract: The present invention relates to novel bacterial isolates identified by their 16S rRNA DNA, that cause periodontal disease in companion animals, polynucleotide sequences contained therein, polypeptides encoded by such polynucleotide sequences and vaccines comprising such bycteria, polynucleotides, or polypeptides. Also provided are methods for trating and preventing periodontal disease and kits for detecting and treating periodontal disease kits for detecting and preventing periodontal disease.

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**VACCINE FOR PERIODONTAL DISEASE****Cross-Reference to Related Application**

This application claims the benefit of U.S. Provisional Patent Application No. 60/342,999 filed December 21, 2001, the contents of which are hereby incorporated by  
5 reference in its entirety.

**Field of the Invention**

The present invention relates to novel bacterial isolates identified by their 16S rRNA DNA, that cause periodontal disease in companion animals, polynucleotide sequences contained therein, polypeptides encoded by such polynucleotide sequences and vaccines  
10 comprising such bacterial isolates that have been inactivated or attenuated, polynucleotides or polypeptides. Also provided are methods for treating and preventing periodontal disease and kits for detecting, treating, and preventing periodontal disease.

**Background Art**

The vast majority of experimental data concerning periodontal diseases is based on  
15 studies of humans or bacteria isolated from humans. Relatively little is known with respect to periodontal disease in non-human animals, such as companion animals, and in particular, dogs and cats.

Periodontal disease comprises a group of infections involving supporting tissues of the teeth. These range in severity from mild and reversible inflammation of the gingiva (gum)  
20 to chronic destruction of periodontal tissues (gingiva, periodontal ligament, and alveolar bone) with eventual exfoliation of teeth.

From a microbiological standpoint, several features of this disease are of interest. The bacterial etiology is complex, with a variety of organisms responsible for the initiation and progression of disease in humans. Many, if not all, of these organisms may also be present in  
25 periodontally healthy individuals and can exist in commensal harmony with the host. Thus, disease episodes may ensue from a shift in the ecological balance between bacterial and host factors, as a result of, for example, alteration in the absolute or relative numbers of certain organisms, changes in pathogenic potential, or modulation of particular host factors. The local environment imposes a variety of unique constraints upon the constituent  
30 microbiota of the supragingival tooth surface and the subgingival crevice (the channel between the tooth root and the gingiva that deepens into a periodontal pocket as disease progresses).

Both the calcified hard tissues of the tooth and the epithelial cells of the gingival are available for colonization. These tissues are exposed to host salivary secretions and gingival  
35 crevicular fluid (a serum exudate), both of which contain molecules that interact directly with bacteria and alter prevailing environmental conditions. In addition, it is known that in humans, successful colonizers of the teeth and subgingival area must coexist with many (over 600) other species of bacteria that inhabit these regions. Study of the pathogenesis of periodontal diseases in humans is thus complicated by the ecological intricacy of the microenvironment.

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The classification of the various manifestations of periodontal disease in humans is continually changing, and it will suffice to mention that diseases range in severity, rate of progression, and number of teeth affected and that different age groups can be susceptible following the eruption of primary teeth. The nature of the pathogenic agents varies among these disease entities, as well as among human patients and even between different disease sites within a patient. In general, however, severe forms of the disease are associated with a number of gram-negative anaerobic bacteria. Of this group, in humans, most evidence points to a pathogenic role for *Porphyromonas* (formerly *Bacteroides*) *gingivalis*. The presence of this organism, acting either alone or as a mixed infection with other bacteria, and possibly in concert with the absence of beneficial species and certain immunological responses in the host, appears to be essential for disease activity.

Colonization of the oral cavity requires that the bacteria first enter the mouth and then localize at and attach to the available surfaces. Host factors which function to prevent bacterial colonization include the mechanical shearing forces of tongue movement along with saliva and gingival crevicular fluid flow. Successful oral colonizers therefore possess a variety of attributes to overcome host protective mechanisms. The sessile plaque biofilm that subsequently accumulates on the hard and soft tissues of the mouth is a dynamic system composed of diverse microbial species. In humans, *P. gingivalis* is usually among the late or secondary colonizers of the oral cavity, requiring antecedent organisms to create the necessary environmental conditions.

Initial entry of *P. gingivalis* into the human oral cavity is thought to occur by transmission from infected individuals. Other vectors would therefore also appear to be operational. These studies indicate that individuals are colonized by a single (or at least a predominant) genotype, regardless of site of colonization or clinical status. Strains of many different clonal origins, in contrast, are present in different individuals. This supports the concept that *P. gingivalis* is essentially an opportunistic pathogen, with virulence not being restricted to a particular clonal type.

The human oral cavity provides a variety of surfaces to which *P. gingivalis* can adhere. There are the mineralized hard tissues of the teeth, along with mucosal surfaces including those of the gingiva, cheek, and tongue.

While a great deal is known about periodontal disease in humans, as described above, very little is known about the same disease in companion animals. Fournier, D. *et al.*, "Porphyromonas gulae sp. nov., an Anaerobic, Gram-negative, Coccibacillus from the Gingival Sulcus of Various Animal Hosts", International Journal of a Systematic and Evolutionary Microbiology (2001), 51, 1179-1189 describe several strains isolated from various animal hosts, including a strain, *P. gulae* spp. nov., designated ATCC 57100. The authors hypothesize that strains for the animal biotype of *P. gingivalis* represent a *Porphyromonas* species that is distinct from *P. gingivalis*. There is no mention of a vaccine useful in treating periodontal disease in companion animals. Hirasawa and Takada, in

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"*Porphyromonas gingivicanis* sp. nov. and *Porphyromonas crevioricanis* sp. nov., Isolated from Beagles", International Journal of Systemic Bacteriology, pp. 637-640, (1994), describe two bacterial species isolated from gingival crevicular fluids of beagles. These species are described in United States Patent Nos. 5,710,039 and US 5,563,063. Nowhere do the authors  
5 suggest the use of these species in a vaccine to treat periodontal disease. International Application PCT/AU98/01023, having publication number WO 99/29870, described various *P. gingivalis* polypeptides and nucleotides. However, no evidence of vaccines effective in preventing periodontal disease in companion animals is provided. Even though there is a great amount of information known about the human disease, little has been accomplished by  
10 way of preventing or treating the disease, even in humans.

There remains a need for a safe and effective vaccine for treating and preventing periodontal disease in companion animals.

#### **Summary of the Invention**

The present invention provides an isolated pigmented anaerobic bacteria having a  
15 16S rRNA DNA sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 94, provided that the bacteria is not a strain of *Porphyromonas gingivalis* designated as dog 20B.

In one embodiment, the bacteria is selected from the group consisting of  
20 *Porphyromonas gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106 and *P. endodontalis* B114, , provided that the bacteria is not a strain of *Porphyromonas gingivalis* designated as dog 20B.

In another embodiment, the present invention provides an isolated pigmented anaerobic bacteria which causes, either directly or in combination with other pathogenic  
25 agents, periodontal disease in companion animals, wherein the bacteria can be used to prepare a vaccine for treating or preventing periodontal disease in companion animals, wherein the vaccine comprises an immunologically effective amount of at least one bacteria which has been inactivated or attenuated, provided that the bacteria is not a strain of *P. gulae* sp. nov. designated ATCC 51700. Preferably, the bacteria has a 16S rRNA DNA sequence at  
30 least about 95% homologous to any of the sequences depicted in SEQ ID NOS: 86 to 94. More preferably, the bacteria has a 16S rRNA DNA sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 94.

In another embodiment, the present invention provides an isolated pigmented anaerobic bacteria which causes, either directly or in combination with other pathogenic  
35 agents, periodontal disease in companion animals, wherein the bacteria can be used to prepare a vaccine for treating or preventing periodontal disease in companion animals, wherein the vaccine comprises an isolated polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, wherein the polypeptide is encoded by a polynucleotide molecule isolated from the bacteria provided that the bacteria

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is not a strain of *P. gulae* sp. nov. designated ATCC 51700. Preferably, the bacteria has a 16S rRNA DNA sequence at least about 95% homologous to any of the sequences depicted in SEQ ID NOS: 86 to 94. More preferably, the bacteria has a 16S rRNA DNA sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 94.

In a further embodiment, the present invention provides an isolated pigmented anaerobic bacteria which causes, either directly or in combination with other pathogenic agents, periodontal disease in companion animals, wherein the bacteria can be used to produce a vaccine for treating or preventing periodontal disease in companion animals, wherein the vaccine comprises an isolated polynucleotide molecule which encodes a polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, wherein the polynucleotide molecule is isolated from the bacteria, provided that the bacteria is not a strain of *P. gulae* sp. nov. designated ATCC 51700. Preferably, the bacteria has a 16S rRNA DNA sequence at least about 95% homologous to any of the sequences depicted in SEQ ID NOS: 86 to 94. More preferably, the bacteria has a 16S rRNA DNA sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 94.

The companion animal is preferably a dog or a cat.

In another aspect, the present invention provides isolated polynucleotide molecule comprising a nucleotide sequence isolated from a bacteria selected from the group consisting of a bacterium having the identifying characteristics of *Porphyromonas gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106 and *P. endodontalis* B114 provided that the bacteria is not a strain of *P. gulae* sp. nov. designated ATCC 51700.

In one embodiment, the isolated polynucleotide molecule is isolated from a bacterium, wherein the bacterium is selected from the group consisting of *Porphyromonas gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106 and *P. endodontalis* B114.

In another embodiment, the isolated polynucleotide according to claim 15 or 16 wherein the polynucleotide encodes for a polypeptide.

In yet another embodiment, the isolated polynucleotide according to claim 15 or 16 wherein, the polynucleotide encodes ribosomal RNA or transfer RNA.

In yet a further embodiment, the present invention provides an isolated polynucleotide molecule comprising any of the nucleotide sequences selected from the group consisting of SEQ ID NOS: 86 to 94 and homologues having at least 95% homology thereto, provided that the nucleotide sequence is not the 16S rRNA DNA from bacteria *P. gulae* sp. nov. designated ATCC 51700.

Preferably, the isolated polynucleotide molecule comprising any of the nucleotide sequences selected from the group consisting of SEQ ID NOS: 95 to 102 and 111-119, (*fimA*

or *oprF*, respectively), which sequence encodes a polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, or complements thereto.

Also preferred is the isolated polynucleotide molecule comprises any of the  
5 nucleotide sequences depicted in SEQ ID NOS: 95 to 102 and 111-119, homologues having at least 95% homology thereto, which sequence encodes a polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, or complements thereto.

In a further embodiment, the isolated polynucleotide molecule comprises any of the  
10 nucleotide sequences depicted in SEQ ID NOS: 95 to 102 and 111-119 or fragments or variants thereof, which sequence encodes a polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, or complements thereto.

In yet a further embodiment, the isolated polynucleotide molecule comprises a  
15 nucleotide sequence which hybridizes under conditions of high stringency to any of the sequences depicted in SEQ ID NOS: 95 to 102 and 111-119, or complements thereto. Preferably, the isolated polynucleotide sequence, wherein said sequence comprises the sequence of *fimA*, selected from any of the sequences depicted in SEQ ID NOS: 95 to 102, a fragment or variant thereof, which fragment or variant has at least about 95%, 98% or 99%  
20 sequence identity thereto. Also preferred is the isolated polynucleotide molecule, wherein said sequence comprises the sequence of *oprF*, selected from, selected from any of the sequences depicted in SEQ ID NOS, 111 to 119, a fragment or variant thereof, which fragment or variant has at least about 95%, 98% or 99% sequence identity thereto.

Preferably, the fragment or variant of the polynucleotide molecule according to the  
25 present invention is at least about 98% homologous thereto.

In another embodiment, the present invention provides an isolated polynucleotide molecule, comprising a nucleotide sequence that hybridizes under conditions of high stringency to *fimA*, selected from any of the sequences depicted in SEQ ID NOS, 95 to 102, or the complement thereof.

In yet another embodiment, the present invention provides isolated polynucleotide  
30 molecule, comprising a nucleotide sequence that hybridizes under conditions of high stringency to *oprF*, selected from any of the sequences depicted in SEQ ID NOS, 111 to 119, or the complement thereof.

The present invention also provides an isolated polynucleotide molecule comprising a  
35 nucleotide sequence of about 30 nucleotides, which hybridizes under highly stringent conditions to a DNA molecule having a nucleotide sequence encoding a polypeptide having a sequence of at least about 10 contiguous amino acids of any of the polypeptides encoded by any of the nucleotide sequences of SEQ ID NOS: 95 to 102 and 109 to 119, or its complement. Preferably, the isolated polynucleotide molecule comprises at least about 90

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nucleotides, which hybridizes under conditions of high stringency to a DNA molecule having a nucleotide sequence encoding a polypeptide having a sequence of at least about 30 contiguous amino acids of any of the polypeptides encoded by any of the nucleotide sequences of SEQ ID NOS: 95 to 102 and 111 to 119, or its complement.

5 In another aspect, the present invention provides the isolated polynucleotide according to the present invention operably linked to a heterologous promoter. The isolated polynucleotide can further comprise an origin of replication active in a prokaryotic or eukaryotic cell.

10 In another aspect, the present invention provides a recombinant expression vector comprising a polynucleotide selected from the group consisting of any of the nucleotide sequences SEQ ID NOS: 95 to 102 and 111 to 119, fragments or variants thereof, operably linked to a promoter sequence.

15 In yet another aspect, the present invention provides a plasmid comprising a polynucleotide selected from the group consisting of any of the nucleotide sequences SEQ ID NOS: 95 to 102 and 111 to 119, fragments or variants thereof, operably linked to a promoter sequence.

In a further aspect, the present invention provides a host cell comprising the isolated polynucleotide sequence, vector or plasmid according to the present invention.

20 Preferably, the host cell is *E. coli* BL21 and said polynucleotide further comprises the expression vector pBAD/HisA or a  $\lambda$  expression plasmid.

In a further aspect, the present invention provides, a method for the production of recombinant FimA or, OprF, selected from any of the sequences depicted in SEQ ID NOS: 103 to 110 or 120 to 128, or fragments or variants thereof, said method comprising (1) growing the cells of claim 36 under conditions in which a polypeptide comprising FimA, OprF, 25 or fragments or variants thereof is expressed, and (2) recovering said polypeptide. The polypeptide can be recovered in soluble or insoluble form.

In another aspect, the isolated polypeptide of the present invention is immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals and comprises an amino acid sequence depicted in SEQ ID NOS: 103 to 110 and 120 to 128.

30 In one embodiment, the isolated polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals comprises an amino acid sequence depicted in SEQ ID NOS: 103 to 110 and 120 to 128 and homologues having at least 95%, 98%, or 99% sequence identity thereto.

35 In another embodiment, the isolated polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals comprises an amino acid sequence depicted in SEQ ID NOS: 103 to 110 and 120 to 128, or fragments or variants thereof.

In yet another embodiment, the isolated polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals having an amino

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acid sequence encoded by a DNA molecule comprises a nucleotide sequence which hybridizes under conditions of high stringency to any of the sequences depicted in SEQ ID NOS: 95 to 102 and 111 to 119.

In yet a further embodiment, the isolated polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, which polypeptide comprises at least about 10 contiguous amino acids comprises a fragment of any of the polypeptide sequences of SEQ ID NOS: 103 to 110 and 120 to 128, which polypeptide is immunologically effective, either alone or linked to a carrier, as a vaccine for preventing or treating periodontal disease in companion animals. Preferably, the isolated polypeptide comprises at least about 25 amino acids.

Preferably, the isolated polypeptide, for preventing or treating periodontal disease in companion animals, encoded by a DNA molecule comprising a nucleotide sequence which comprises the sequence of *fimA* (SEQ ID NOS: 95 to 102).

Also preferred, the isolated polypeptide, for preventing or treating periodontal disease in companion animals, encoded for by a DNA molecule comprising a nucleotide sequence which comprises the sequence of *oprF* (SEQ ID NOS: 111 to 119).

In a preferred embodiment, the isolated polypeptide is a recombinantly expressed polypeptide, which polypeptide is selected from the group consisting of FimA (SEQ ID NOS: 103 to 110) and OprF (SEQ ID NOS: 120 to 128).

In another embodiment, the recombinantly expressed polypeptide is fused to a carrier polypeptide. The fusion polypeptide is preferably essentially a poly-histidine or poly-threonine sequence.

In a further aspect, the present invention provides a vaccine for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one inactivated pigmented anaerobic bacteria according to the present invention, and a pharmaceutically acceptable carrier.

In another aspect, the present invention provides a vaccine for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one polynucleotide molecule according to the present invention, and a pharmaceutically acceptable carrier.

In yet another aspect, the present invention provides vaccine for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one polypeptide according to the present invention, and a pharmaceutically acceptable carrier.

Preferably, the vaccine for treating or preventing periodontal disease in companion animals comprises an immunologically effective amount of FimA and a pharmaceutically acceptable carrier.



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Also preferred is a vaccine for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of OprF and a pharmaceutically acceptable carrier.

5 The bacteria for use in the vaccines of the present invention may be selected from the group consisting of *Porphyromonas gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106 and *P. endodontalis* B114.

10 In still another embodiment, the present invention provides a vaccine composition for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one inactivated isolated pigmented anaerobic bacteria according to the present invention, a pharmaceutically acceptable carrier, and optionally an adjuvant.

15 In yet another embodiment, the present invention provides a vaccine composition for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one polynucleotide molecule according to the present invention, a pharmaceutically acceptable carrier, and optionally, an adjuvant.

20 In still a further embodiment, the present invention provides a vaccine composition for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one polypeptide according to the present invention, a pharmaceutically acceptable carrier, and optionally, an adjuvant.

In another aspect the present invention provides a method for treating or preventing periodontal disease in companion animals comprising administering to a companion animal in need thereof, a vaccine composition according to the present invention.

25 In another aspect the present invention provides a method for diagnosing periodontal disease in companion animals by analyzing a sample for bacteria, polypeptides or polynucleotides of the present invention, wherein the presence of the bacteria, polypeptides, or polynucleotides are indicative of disease. Preferably, the analyzing step includes analyzing the sample using a method selected from the group consisting of PCR, hybridization, and antibody detection.

30 In yet another aspect, the present invention provides a kit comprising, in at least one container, a composition for treating and preventing periodontal disease in companion animals comprising an effective amount of at least one inactivated isolated pigmented anaerobic bacteria, polypeptide, or polynucleotides of the present invention and a pharmaceutically acceptable carrier; wherein the kit further comprises a set of printed  
35 instructions indicating that the kit is useful for treating or preventing periodontal disease in companion animals. The kit may further comprises a means for dispensing said composition.

In still another aspect, the present invention provides a kit comprising in at least one container an isolated DNA molecule comprising a nucleotide sequence of at least about 15 contiguous nucleotides selected from any of SEQ ID NOS: 86 to 94, 95 to 102, and 111 to

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119 which hybridizes under highly stringent conditions to the complement of any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119, and a second isolated DNA molecule comprising in a second container an isolated DNA molecule comprising a nucleotide sequence of at least about 15 contiguous nucleotides selected from the complement of any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119 which hybridizes under highly stringent conditions to any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119, wherein the kit further comprises a set of instructions indicating that the kit is useful for the detection of *Porphyromonas* spp. Such a method may be used generally in all mammals, including humans.

In yet another aspect, the present invention provides a kit comprising in at least one container a protein having an amino acid sequence comprising at least 30 contiguous amino acids, which polypeptide is encoded by any of the nucleotide sequences of SEQ ID NOS: 95 to 102 and 111 to 119 and a statement indicating that the kit is useful for the detection of *Porphyromonas* spp. The kit may further comprise a second polypeptide, wherein the second polypeptide is an antibody which is conjugated to an enzyme that catalyzes a colorimetric or The enzyme is preferably selected from the group consisting of alkaline phosphatase and horseradish peroxidase. The kit may further comprise reagents for a colorimetric or chemiluminescent assay.

In a further aspect, the present invention provides a hybridization kit comprising in at least one container an isolated DNA molecule comprising a nucleotide sequence of at least about 15 contiguous nucleotides selected from any of SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119, or its complement, wherein the hybridization is specific to *Porphyromonas* spp. and wherein the kit further comprises a set of instructions indicating that the kit is useful for the detection of *Porphyromonas* spp. Preferably, the hybridization is performed under highly stringent conditions.

None of the bacteria, polynucleotides, polypeptides, vaccine, vaccine compositions or kits of the present invention comprise any of the bacteria, polynucleotides or peptides described in Fournier, D. et al., "*Porphyromonas gulae* sp. nov., an Anaerobic, Gram-negative, Coccibacillus from the Gingival Sulcus of Various Animal Hosts", International Journal of a Systematic and Evolutionary Microbiology (2001), 51, 1179-1189, including a strain, *P. gulae* spp. nov., designated ATCC 57100, Hirasawa and Takada, "*Porphyromonas gingivicanis* sp. nov. and *Porphyromonas crevioricanis* sp. nov., Isolated from Beagles", International Journal of Systemic Bacteriology, pp. 637-640, (1994), United States Patent Nos. 5,710,039 or US 5,563,063, or International Application PCT/AU98/01023, having publication number WO 99/29870.

#### **Brief Description of the Figures**

Figure 1 is a graph showing the results of a growth study identifying an "animal product-free" medium that supports the growth of *Porphyromonas gulae* B43. The following

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medium were tested: ME-complete, ME-hemin, ME-vitamin K, ME-both hemin and vitamin K, PYG-complete, PYG-hemin, PYG-vitamin K, PYG-both hemin and vitamin K, and BHI.

Figure 2 is a graph showing mean bone loss in mice resulting from super infection with the indicated *Porphyromonas* sp.

5        Figure 3 is a graph showing percent bone loss in mice resulting from super infection with the indicated *Porphyromonas* sp.

Figures 4A and B are photographs showing in Figure 4A, an SDS PAGE, and in Figure 4B a Western blot analysis, using the anti-Xpress™ epitope serum (Invitrogen), of recombinant *P. gulae* B43 FimA expressed in *E. coli* BL21 from pBAD-HisA.

10       Figure 5 is a photograph showing SDS-PAGE analysis of recombinant *P. gulae* B43 OprF expressed in *E. coli* BL21 cells from a lambda expression plasmid.

Figure 6 is a graph showing the results of a homologous vaccine efficacy study based upon net bone loss;

15       Figure 7 is a graph showing a *P. gingivalis* 53977 homologous vaccine efficacy study based upon percent bone loss.

Figure 8 is a graph showing a *P. gulae* B43 homologous vaccine efficacy study based upon percent bone loss.

Figure 9 is a graph showing the results of a heterologous vaccine efficacy study based upon net bone loss.

20       Figure 10 is a graph showing the results for *P. gulae* B43 challenge groups of the heterologous vaccine efficacy study based upon percent bone loss.

Figure 11 is a graph showing the results for *P. gulae* B69 challenge groups of the heterologous vaccine efficacy study based upon percent bone loss.

25       Figure 12 is a graph showing the results for *P. salivosa* B104 challenge groups of the heterologous vaccine efficacy study based upon percent bone loss;

Figure 13 is a graph showing the results for *P. denticanis* B106 challenge groups of the heterologous vaccine efficacy study based upon percent bone loss.

Figure 14 is a graph showing the serological results of mice vaccinated with recombinant *P. gulae* B43 FimA or saline utilizing a FimA specific ELISA.

30       Figure 15 is a graph showing the serological results of mice vaccinated with recombinant *P. gulae* B43 OprF or saline utilizing an OprF specific ELISA.

### **Detailed Description of the Invention**

#### **Bacterial Isolates**

35       The present invention provides isolated anaerobic bacteria, identified by their 16S rRNA DNA sequences, which cause periodontal disease and various other diseases and clinical manifestations in companion animals. More specifically, the bacteria are selected from the genus *Porphyromonas*.

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Preferably, the isolated bacteria of the present invention include *P. gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106, and *P. endodontalis* B114, although other species or strains are encompassed by the invention. In a preferred embodiment, the isolated bacteria of the present invention can be identified by their 16S rRNA DNA sequences shown in SEQ ID Nos. 86 to 94.

The diseases caused by infection with the bacteria of the present invention include, but are not limited to, companion animal periodontal disease, companion animal oral malodor (halitosis), bovine foot rot, canine coronary heart disease and canine systemic infections. Bacteria within the genus *Porphyromonas* have also been connected with various human diseases, including coronary heart disease, parotitis, oral malodor, gingivitis, periodontitis, stroke, atherosclerosis, hyperlipidemia, bacterial vaginosis, intrauterine growth retardation (IUGR), and increased incidence of pre-term delivery of low birth weight infants.

The present invention provides isolated polynucleotide and isolated polypeptide molecules of *Porphyromonas* spp. More particularly, the invention provides isolated polynucleotide molecules having the nucleotide sequence of *Porphyromonas* spp. *fimA* and *oprF* genes or degenerate variants thereof and isolated polypeptide molecules having the amino acid sequences of the FimA and OprF proteins encoded by such genes, respectively.

The present invention also provides polynucleotide sequences having at least about 90% homology, preferably at least about 95%, and most preferably at least 99%, sequence identity to any of SEQ ID NOS: 95 to 102 and 111 to 119 as determined using any known standard identity algorithm. In addition, the present invention provides polynucleotide sequences that hybridize under stringent conditions to the complement of any of the polynucleotide sequences shown in SEQ ID NOS: 95 to 102 and 111 to 119.

In another specific embodiment, a nucleic acid which is hybridizable to any of the polynucleotide sequences depicted in SEQ ID No. 86 to 102 and 111 to 119, or their complements, under conditions of high stringency is provided. By way of example and not limitation, procedures using such conditions of high stringency for regions of hybridization of over 90 nucleotides are as follows. Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/mL denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture containing 100 µg/mL denatured salmon sperm DNA and 5-20 X 10<sup>6</sup> cpm of <sup>32</sup>P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography.

Other conditions of high stringency which may be used depend on the nature of the nucleic acid (e.g. length, GC content, etc.) and the purpose of the hybridization (detection, amplification, etc.) and are well known in the art. For example, stringent hybridization of an oligonucleotide of approximately 15-40 bases to a complementary sequence in the

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polymerase chain reaction (PCR) is done under the following conditions: a salt concentration of 50 mM KCl, a buffer concentration of 10 mM Tris-HCl, a  $Mg^{2+}$  concentration of 1.5 mM, a pH of 7-7.5 and an annealing temperature of 55-60°C.

In a preferred specific embodiment, after hybridization, wash conditions are as follows. Each membrane is washed two times each for 30 minutes each at 45°C in 40 mM sodium phosphate, pH 7.2, 5% SDS, 1 mM EDTA, 0.5% bovine serum albumin, followed by four washes each for 30 minutes in sodium phosphate, pH 7.2, 1% SDS, 1 mM EDTA. For high stringency hybridization, the membranes are additionally subjected to four washes each for 30 minutes in 40 mM sodium phosphate, pH 7.2, 1% SDS, 1 mM EDTA at 55°C, followed by four washes each for 30 minutes in sodium phosphate, pH 7.2, 1% SDS, 1 mM EDTA at 65°C.

The present invention further provides vaccines and vaccine formulations which, when administered to a companion animal in a therapeutically effective amount, are useful in treating or preventing (i.e., conferring resistance) to periodontal disease in a companion animal.

In one embodiment, the present invention provides a vaccine that comprises at least one attenuated (modified live) or inactivated whole cell *Porphyromonas* spp. preparation (bacterin). In another embodiment, the vaccine comprises a subunit fraction of a *Porphyromonas* spp. capable of inducing an immune response.

In a preferred embodiment the vaccine of the present invention comprises one or more subunit polypeptides or fragments or variants thereof, or one or more isolated polynucleotide sequences or fragments or variants thereof.

The attenuated (modified live) or inactivated vaccines (bacterins), or isolated subunit polypeptides, or isolated polynucleotides can be present in combination with other known vaccine formulation components such as with compatible adjuvants, diluents, or carriers.

#### **Definitions and Abbreviations**

The term "ORF" indicates "open reading frame", i.e. the coding region of a gene.

The term "Percentage of sequence identity" for nucleotide sequences and polypeptide sequences is determined by comparing two optimally aligned sequences over a comparison window, wherein optimal alignment provides the highest order match and can introduce nucleotide or amino acid additions or to the test or reference sequence. The percentage identity is determined by calculating the percentage of nucleotides- that are identical between the test and reference sequence at each position over the entire sequence. Optimal sequence alignment and percentage identity can be determined manually, or more preferably by a computer algorithm including but not limited to TBLASTN, BLASTP, FASTA, TFASTA, GAP, BESTFIT, and CLUSTALW (Altschul et al., 1990, J. Mol. Biol. 215(3):403-10; Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85(8):2444-8; Thompson, et al., 1994, Nucleic Acids Res. 22(22):4673-80; Devereux et al., 1984, Nuc. Acids. Res. 12:387-395; Higgins, et al., 1996, Methods Enzymol. 266:383-402). Preferably, the NCBI Blast Server

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(<http://www.ncbi.nlm.nih.gov>) set at the default parameters is used to search multiple databases for homologous sequences.

The term "heterologous", when used herein means derived from a different bacterial species or strain.

5       The term "homology ", "homologous", and the like, when used herein means the degree of identity shared between polynucleotide or polypeptide sequences.

The term "homologous", when used in reference to a bacterial species means the same bacterial species or strain.

10       The term "host cell", when used herein means a bacteria or eukaryotic cell that harbors a plasmid, virus, or other vector.

The term "isolated" when used herein means removed from its naturally occurring environment, either alone or in a heterologous host cell, or chromosome or vector (e.g., plasmid, phage, etc.).

15       The terms "isolated anaerobic bacteria", "isolated bacteria", "isolated bacterial strain" and the like refer to a composition in which the bacteria are substantially free of other microorganisms, e.g., in a culture, such as when separated from its naturally occurring environment.

20       The term "isolated polynucleotide" indicates a composition in which the isolated nucleotide comprises at least 50% of the composition by weight. More preferably, the isolated polynucleotide comprises about 95%, and most preferably 99% by weight of the composition.

25       The term "isolated polypeptide" indicates a composition in which the isolated polypeptide comprises at least 50% of the composition by weight. More preferably, the isolated polypeptide comprises about 95%, and most preferably 99% by weight of the composition.

30       The term "functionally equivalent" as utilized herein, refers to a recombinant polypeptide capable of being recognized by an antibody specific to native polypeptide produced by the bacteria which causes periodontal disease in companion animals, or a recombinant polypeptide capable of eliciting or causing a substantially similar immunological response as that of the native protein from the endogenous bacteria. Thus, an antibody raised against a functionally equivalent polypeptide also recognizes the native polypeptide produced by the bacteria which causes periodontal disease in companion animals.

35       The term "immunogenicity" refers to the capability of a protein or polypeptide to elicit an immune response directed specifically against the bacteria that causes periodontal disease in companion animals.

The term "antigenicity" refers to the capability of a protein or polypeptide to be immunospecifically bound by an antibody raised against the protein or polypeptide.

The term "antibody", as used herein, refers to an immunoglobulin molecule able to bind to an antigen. Antibodies can be a polyclonal mixture or monoclonal. Antibodies can be

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intact immunoglobulins derived from natural sources or from recombinant sources, or can be immunoreactive portions of intact immunoglobulins. Antibodies can exist in a variety of forms including, for example, as, Fv, Fab', F(ab')<sub>2</sub>, as well as in single chains.

5 The term "companion animal", as used herein, refers to any non-human animal in captivity considered to be a pet. These may include, but are not restricted to, dogs, cats, horses, rabbits, monkeys, and rodents, including mice, rats, hamsters, gerbils, and ferrets.

The term "protection", "protecting", and the like, as used herein with respect to a vaccine, means that the vaccine prevents or reduces the symptoms of the disease caused by the organism from which the antigen(s) used in the vaccine is derived. The terms "protection" and "protecting" and the like, also mean that the vaccine can be used to "treat" the disease or one of more symptoms of the disease that already exists in a subject.

10 The term "therapeutically effective amount" refers to an amount of the bacteria, or a subunit, (e.g., polypeptides, polynucleotide sequences) and combinations thereof sufficient to elicit an immune response in the subject to which it is administered. The immune response can comprise, without limitation, induction of cellular and/or humoral immunity.

15 The term "preventing infection" means to prevent or inhibit the replication of the bacteria which cause periodontal disease in companion animals, to inhibit transmission of the bacteria, or to prevent the bacteria from establishing itself in its host, or to alleviate the symptoms of the disease caused by infection. The treatment is considered therapeutic if there is a reduction in bacterial load.

20 The term "pharmaceutically acceptable carrier" refers to a carrier medium that does not interfere with the effectiveness of the biological activity of the active ingredient and is not toxic to the subject to whom it is administered.

The term "therapeutic agent" refers to any molecule, compound or treatment, preferably an antibacterial, that assists in the treatment of a bacterial infection or a disease or condition caused thereby.

25 The term "fragment or variant thereof" refers to partial nucleotide or amino acid sequences according to the present invention. Preferably the fragments or variants of the polypeptides that are provided in the present invention are capable of eliciting a humoral and/or cellular immune response in a companion animal. Analogs are encompassed by the term "fragment or variant thereof". Mutant polynucleotides which may possess one or more mutations which are deletions, insertions or substitutions of nucleotide residues are encompassed by the term "fragment or variant thereof". Allelic variants are encompassed by the term "fragment or variant thereof".

### 35 **Isolation and Characterization of *Porphyromonas* spp.**

Bacteria provided by the present invention can be obtained using known sampling, culture and isolation techniques. For example, microbial samples can be obtained from a population of companion animals, such as from dogs and cats, exhibiting periodontal disease. Evidence of periodontal disease can be observed using known measures, such as dogs with

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periodontal pockets >3mm and cats with periodontal pockets >2mm. Known parameters for characterizing periodontal disease such as dental indices (gingival index and periodontal index) and periodontal pocket depths can be determined for the sample population of companion animals. Individual samples can be obtained from the periodontal pocket of a particular animal, maintained under anaerobic conditions and cultured using various known culture media.

Clinical isolates can be characterized using known techniques such as a number of biochemical tests, and 16S rRNA DNA sequence analysis to determine their genus and species. Individual isolates can be transferred to plates and antibiotic disks (Anaerobe Systems) can be placed on the agar surface to determine the antibiotic resistance patterns of each isolate. Purified colonies can also be subjected to known indole and catalase tests (Anaerobe Systems). Lipase and lecithinase production patterns can be determined for individual isolates.

The isolates can be typed based on their 16S rRNA DNA sequence. Individual, well-isolated colonies can be utilized as a template for polymerase chain reactions (PCR) amplification of the 16S rRNA region using, for example, primers D0056 and D0057 (Seq. ID NO. 1 and Seq. ID NO. 2; Table 1). The resulting PCR products can be purified using available PCR preps kits (Promega Corp.; Madison, WI) and pooled by isolate. The purified PCR products can then be desalted and subjected to DNA sequence analysis. The resulting DNA sequences can be used to search available DNA databases. The bacterial isolates can then be typed based on the closest match identified by database searches.

**Table 1.** DNA sequence identification listing. All oligonucleotide primers were synthesized by either Gibco-BRL (USA) or Lark Technologies Inc. (USA).

SEQ ID NO.	Name	Target	DNA Sequence
1	D0056	16S rRNA	GGATTAGATACCCTGGTAGTC
2	D0057	16S rRNA	CCCGGGAACGTATTCACCG
3	PFZ175-AP1	16S rRNA	GGCTTAAGTGCCATAACGAG
4	PFZ175-AP2	16S rRNA	CTGGCGTCTTACGACGGCTG
5	PFZ175-AP3	16S rRNA	TGTCGTCAGCTCGTGCCGTG
6	D0067	<i>fimA</i>	GCGCAGCAAGGCCAGCCCGG
7	D0068	<i>fimA</i>	GAGCGAACCCGCTCCCTGT
8	D0078	<i>fimA</i>	GCGACGCTATATGCAAGACAATC
9	D0097	<i>fimA</i>	ggcctcgagAACAAAGACAACGAAGCAGAACC
10	D0098	<i>fimA</i>	ggcaagcttACCAAATAACATTTTGTACAACAAC
11	PFZ185-AP1	<i>fimA</i>	TCATCCGACAATCCTGTGTG
12	PFZ185-AP2	<i>fimA</i>	AGCAGCTGCTAAATCGGCTC



SEQ ID NO.	Name	Target	DNA Sequence
13	PFZ185-AP3	<i>fimA</i>	TTGGCAAGACTCTTGCAGAG
14	PFZ185-AP4	<i>fimA</i>	CTGCAGTCAGTTCAGTTGTC
15	PFZ186-AP1	<i>fimA</i>	TACGTCAACAGGCTCTGCTG
16	PFZ186-AP2	<i>fimA</i>	GACAACTGAACTAACTGCAG
17	PFZ186-AP3	<i>fimA</i>	AACATAGAAACCTTGTGGAG
18	PFZ186-AP4	<i>fimA</i>	TGTCGTCTGGTTGGGAAGAG
19	PFZ186-AP5	<i>fimA</i>	AATCTGATTGCCTCCCTGAG
20	PFZ187-AP1	<i>fimA</i>	GGGAACCGATTTAGCAGCAG
21	PFZ187-AP2	<i>fimA</i>	CCAATACAGGGTAATAGGTC
22	PFZ187-AP3	<i>fimA</i>	GTTGTCAATGCTTTTACCTC
23	PFZ187-AP4	<i>fimA</i>	GATTGAGAATATCAAATGTG
24	PFZ187-AP5	<i>fimA</i>	TTAGGCGTATAACCATTGTC
25	PFZ187-AP6	<i>fimA</i>	ATTTAACGGTGCTTACACAC
26	PFZ187-AP7	<i>fimA</i>	CCAATTGGCGGCCTGAGCTG
27	PFZ187-AP8	<i>fimA</i>	TGGCATAGTTGGTAGGTGTG
28	PFZ187-AP9	<i>fimA</i>	TGTAAGCACCGTTAAATGTG
29	PFZ187-AP11	<i>fimA</i>	CTGACAGGTTCTTTGACCAC
30	PFZ187-AP12	<i>fimA</i>	TGTTCCTTGGTTGAGCCGTG
31	PFZ187-AP13	<i>fimA</i>	GTGGTCAAAGAACCTGTCAG
32	PFZ187-AP14	<i>fimA</i>	CATAAACACACAGGATTGTC
33	PFZ187-AP15	<i>fimA</i>	TTGCTTCTTTGCAATGAGAC
34	PFZ187-AP16	<i>fimA</i>	AGCCATGCGAGCATGTACAC
35	PFZ187-AP17	<i>fimA</i>	CTGTCATGATCAAACCTGTG
36	PFZ187-AP18	<i>fimA</i>	ACCGTCTGCATTCACGAGTG
37	PFZ188-AP1	<i>fimA</i>	GCCTTCCAATGATGCTCCAC
38	PFZ188-AP2	<i>fimA</i>	GGACGTAGACCTGCATTCTG
39	PFZ188-AP3	<i>fimA</i>	CGCAATACGGGCATGAACAC
40	PFZ188-AP4	<i>fimA</i>	TTATGGTTATGATGGACCTC
41	PFZ188-AP5	<i>fimA</i>	TGGTACTCCTTTGAGTTCTG
42	PFZ188-AP6	<i>fimA</i>	CACACTTGCGCGGTAACCAC
43	D0086	<i>oprF1</i>	ATGAAGGTAAAGTACTTAATGC
44	D0087	<i>oprF1</i>	AGATGAATTACTTGGAGCGAACGAT
45	KWK-Pg-03	<i>oprF1</i>	TTACTTGGAGCGAACGATTACAACACG
46	PFZ209-AP1	<i>oprF1</i>	TTGGTGCAGCTCACTTCGAC
47	PFZ209-AP2	<i>oprF1</i>	ACCACATCAAACATAAAGTC
48	PFZ209-AP3	<i>oprF1</i>	ACATTCGGGGCATGATACAG

SEQ ID NO.	Name	Target	DNA Sequence
49	PFZ209-AP4	<i>oprF1</i>	ATGCCATTGAGCCAATGGAC
50	PFZ210-AP1	<i>oprF1</i>	TTGACTTCATGTTTCGATGTG
51	PFZ210-AP2	<i>oprF1</i>	TGCCAATGAATTTTATGCTG
52	PFZ210-AP3	<i>oprF1</i>	CGCTTGGAGAGTTCTTCGAC
53	PFZ210-AP4	<i>oprF1</i>	TATCAACGATCTGAATGGTC
54	PFZ211-AP1	<i>oprF1</i>	AACTACTTCAAGCCCTACAG
55	PFZ211-AP2	<i>oprF1</i>	CGTAACCCAAACCTACCCAC
56	PFZ211-AP3	<i>oprF1</i>	ACGGGACGCTTGCTCAACTC
57	PFZ211-AP4	<i>oprF1</i>	ATTGGGGCTTGGTAAATGAC
58	PFZ211-AP5	<i>oprF1</i>	ATACGCTCTACACGAGGCTC
59	PFZ212-AP1	<i>oprF1</i>	CCGCCATGGCTGGAGCTCAC
60	PFZ212-AP2	<i>oprF1</i>	TTTGAAACCATATCCCACAC
61	PFZ212-AP3	<i>oprF1</i>	AGTAACTTCAGGACATTCTG
62	PFZ212-AP4	<i>oprF1</i>	ACGTCCAGTTTCTTGCCCAG
63	PFZ213-AP1	<i>oprF1</i>	TTGACTTCATGTTTCGATGTG
64	PFZ213-AP2	<i>oprF1</i>	TTTGTGTTGGTAACCAACAC
65	PFZ213-AP3	<i>oprF1</i>	ACAGGACGCTTAGAGAGCTC
66	PFZ213-AP4	<i>oprF1</i>	ACGCGCTTATCAACGATCTG
67	PFZ213-AP5	<i>oprF1</i>	CTTCCCAAGGAACGTGTGTG
68	PFZ214-AP1	<i>oprF1</i>	ACTTTATGTTTGATGTTGTG
69	PFZ214-AP2	<i>oprF1</i>	CCAACACCGAACCAAGGCAC
70	PFZ214-AP3	<i>oprF1</i>	TCTCAACTCAGTATTCTCAG
71	PFZ214-AP4	<i>oprF1</i>	TAACCTTAATTTTGGTCGTG
72	PFZ215-AP1	<i>oprF1</i>	CACACCTACAACACTGCCAC
73	PFZ215-AP2	<i>oprF1</i>	TCAAACATGAAATCATAGTG
74	PFZ215-AP3	<i>oprF1</i>	CTCGGGGCAGAAAGCAGGAC
75	PFZ215-AP4	<i>oprF1</i>	GACTTGAACCTCTCAGATCAG
76	KWK-Pg-06	<i>oprF1</i>	atgCAGGAAAATACTGTACCGGCAACG
77	KWK-Pgu-14	<i>oprF1</i>	gtgtgtcatatgCAGGAAAATACTGTACC
78	KWK-Pgu-15	<i>oprF1</i>	gtgtgttagattaTTACTTGGAGCGAACG
79	KWK-Ps-02	<i>oprF1</i>	ACACCTGAGACTCAGACATTGC
80	KWK-Ps-03	<i>oprF1</i>	CATGCGCGAGCCTCAAAAAGC
81	KWK-Ps-04b	<i>oprF1</i>	CCTGCCACTCAACAGAAATCATATCAGAA GGAACCTCC
82	KWK-Ps-05b	<i>oprF1</i>	CTGCTCATAAGACGGCTTTTGACCGTTCT GCAGG

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SEQ ID NO.	Name	Target	DNA Sequence
83	KWK-Ps-06b	<i>oprF1</i>	CTTTTGACCGTTCTGCAGGACATTGGTTC TTGACTCTCC
84	D122	<i>fimA</i>	TGGCTAARYTGACYGTAATGGTYTA
85	D123	<i>fimA</i>	AGTTYACYAATACAGGRTAATAGGT
86	<i>P. gulae</i> B43 16S rRNA polynucleotide sequence	NA	CACGCAGTAAACGATGATTACTAGGAGT TTGCGATATACCGTCAAGCTTCCACAGC GAAAGCGTTAAGTAATCCACCTGGGGAG TACGCCGGCAACGGTGAAACTCAAAGGA ATTGACGGGGGCCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGA GGAACCTTACCCGGGATTGAAATGTAGA CGACGGATGGTGAAAGCCGTCTTCCCTT CGGGGCGTCTATGTAGGTGCTGCATGGT TGTCGTCAGCTCGTGCCGTGAGGTGTCTG GCTTAAGTGCCATAACGAGCGCAACCCA CATCGGTAGTTGCTAACAGGTTTAGCTG AGGACTCTACCGAGACTGCCGTCGTAAG GCGCGAGGAAGGTGTGGATGACGTCAA ATCAGCACGGCCCTTACATCCGGGGCGA CACACGTGTTACAATGGGAGGGACAAAG GGCAGCTACCGGGCGACCGGGTGCGAA TCTCGAAACCCTTCCCCAGTTCGGATCG GAGTCTGCAACTCGACTCCGTGAAGCTG GATTGCTAGTAATCGCGCATCAGCCAT GGCGCGGTGAATAC
87	<i>P. cansulci</i> B46 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGATTACTCGGAGT ATGCGATATGAGTGTATGCTTCTTAGCGA AAGCGTTAAGTAATCCACCTGGGGAGTA CGTCGGCAACGATGAAACTCAAAGGAAT TGACGGGGGCCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAG GAACCTTACCCGGGATTGAAATATAGAT GACAGGCAGCGAGAGTTGTTATCCCTTC GGGGCATCTATGTAGGTGCTGCATGGTT GTCGTCAGCTCGTGCCGTGAGGTGTCTG GCTTAAGTGCCCTAACGAGCGCAACCCA CATTATTAGTTACTAACAGGTTAAGCTGA GGACTCTAATAAGACTGCCGGCGTAAGC

SEQ ID NO.	Name	Target	DNA Sequence
			CGTGAGGAAGGTGTGGATGACGTCAAAT CAGCACGGCCCTTACATCCGGGGCGAC ACACGTGTTACAATGGTAGGGACAAAGG GCAGCTACCGGGCGACCGGATGCGAAT CTCCAAACCCTATCCCAGTTCGGATCGG AGTCTGCAACTCGACTCTGTGAAGCTGG ATTCGCTAGTAATCGCGCATCAGCCATG GCGCGGTGAATAC
88	<i>P. circumdentaria</i> B52 16S rRNA polynucleotide sequence	NA	CACGCTGTAAACGATGAATACTAGATTTT TGCGATATACAGTAAGAGTCTAAGCGAA AGCGATAAGTATTCCACCTGGGGAGTAC GCCGGCAACGGTGAAACTCAAAGGAATT GACGGGGGCCCCGCACAAGCGGAGGAAC ATGTGGTTTAATTTCGATGATACGCGAGG AACCTTACCTGGGATTGAAATTTAGGAGA ACGATTTATGAAAGTAGATTTTCCCTTCG GGGCTCCTAAGTAGGTGCTGCATGGTTG TCGTCAGCTCGTGCCGTGAGGTGTCGGC TTAAGTGCCATAACGAGCGCAACCCGCG TTGATAGTTACTAACAGATAAAGCTGAGG ACTCTATCGAGACAGCCGTCGTAAGACG CGAGGAAGGGGCGGATGACGTCAAATC AGCACGGCCCTTACATCCAGGGCGACAC ACGTGTTACAATGGCAAGGACAAAGGGA AGCCACATAGCGATATGGAGCAGATCCT CAAACCTTGTCCCAGTTCGGATCGGAGT CTGCAACTCGACTCCGTGAAGCTGGATT CGCTAGTAATCGCGCATCAGCCATGGCG CGGTGAATACC
89	<i>P. guiae</i> B69 16S rRNA polynucleotide sequence	NA	CACGCAGTAAACGATGATTACTAGGAGT TTGCGATATACCGATAAGCTTCCACAGC GAAAGCGTTAAGTAATCCACCTGGGGAG TACGCCGGCAACGGTGAAACTCAAAGGA ATTGACGGGGGCCCCGCACAAGCGGAGG AACATGTGGTTTAATTTCGATGATACGCGA GGAACCTTACCCGGGATTGAAATGTAGA TGACAGATGGTGAAAGCCGTCTTCCCTT CGGGGCGTCTATGTAGGTGCTGCATGGT

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SEQ ID NO.	Name	Target	DNA Sequence
			TGTCGTCAGCTCGTGCCGTGAGGTGTCTG GCTTAAGTGCCATAACGAGCGCAACCCA TATCGGTAGTTGCTAACAGGTCAAGCTG AGGACTCTACCGAGACTGCCGTCTAAG GCGAGAGGAAGGTGTGGATGACGTCAA TCAGCACGGCCCTTACATCCGGGGCGAC ACACGTGTTACAATGGGAGGGACAAAGG GCAGCTACCGGGCGACCGGATGCGAAT CTCGAAACCCTTCCCCAGTTCGGATCGG AGTCTGCAACTCGACTCCGTGAAGCTGG ATTCGCTAGTAATCGCGCATCAGCCATG GCGCGGTGAATACC
90	<i>P. circumdentaria</i> B97 16S rRNA polynucleotide sequence	NA	CACGCTGTAAACGATGAATACTAGATTTT TGCGATATACAGTAAGAGTCTAAGCGAA AGCGATAAGTATTCCACCTGGGGAGTAC GCCGGCAACGGTGAAACTCAAAGGAATT GACGGGGGCCCGCACAAAGCGGAGGAAC ATGTGGTTTAATTCGATGATACGCGAGG AACCTTACCTGGGATTGAAATTTAGGAGA ACGATTTATGAAAGTAGATTTTCCCTTCG GGGCTCCTAAGTAGGTGCTGCATGGTTG TCGTCAGCTCGTGCCGTGAGGTGTCTGGC TTAAGTGCCATAACGAGCGCAACCCGCG TCGATAGTTACTAACAGGTAATGCTGAG GACTCTATCGAGACAGCCGTCGTAAGAC GAGAGGAAGGGGCGGATGACGTCAAAT CAGCACGGCCCTTACATCCAGGGCGACA CACGTGTTACAATGGCAAGGACAAAGGG AAGCCACATAGCGATATGGAGCAGATCC TCAAACCTTGTCCCAGTTCGGATCGGAG TCTGCAACTCGACTCCGTGAAGCTGGAT TCGCTAGTAATCGCGCATCAGCCATGGC GCGGTGAATAC

SEQ ID NO.	Name	Target	DNA Sequence
91	<i>P. cangingivalis</i> B98 16S rRNA polynucleotide sequence	NA	CAGTAAACGATGATTACTCGGAGTATGC GATATATGGTATGCTCCCAAGGGAAACC GATAAGTAATCCACCTGGGGAGTACGCC GGCAACGGTGAAACTCAAAGGAATTGAC GGGGGCCCCGCACAAGCGGAGGAACATG TGGTTTAATTTCGATGATACGCGAGGAAC CTTACCCGGGATTGAAATGTACATGACG GTTGGGCGAGAGCCTGACTTCCCTTCGG GGCATGTATGTAGGTGCTGCATGGTTGT CGTCAGCTCGTGCCGTGAGGTGTCGGCT TAAGTGCCATAACGAGCGCAACCCACAT CGTCAGTTACTAACAGGTAGAGCTGAGG ACTCTGACGAGACTGCCGTGTAAGGCG CGAGGAAGGTGTGGATGACGTCAAATCA GCACGGCCCTTACATCCGGGGCGACAC ACGTGTTACAATGGTAGGGACAAAGGGC AGCTACCTGGCGACAGGATGCGAATCTC CAAACCCTATCTCAGTTCGGATCGGAGT CTGCAACTCGACTCCGTGAAGCTGGATT CGCTAGTAATCGCGCATCAGCCATGGCG CGGTGAATACGTT
92	<i>P. salivosa</i> B104 16S rRNA polynucleotide sequence	NA	CAGTAAACGATGATAACTGGGCGTATGC GATATACAGTATGCTCCTGAGCGAAAGC GTTAAGTTATCCACCTGGGGAGTACGCC GGCAACGGTGAAACTCAAAGGAATTGAC GGGGGCCCCGCACAAGCGGAGGAACATG TGGTTTAATTTCGATGATACGCGAGGAAC CTTACCCGGGATTGAAATTTAGCGGACT ATGTATGAAAGTACATATCCTGTCACAAG GCCGCTAAGTAGGTGCTGCATGGTTGTC GTCAGCTCGTGCCGTGAGGTGTCGGCTT AAGTGCCATAACGAGCGCAACCCACGTT GTCAGTTACTATCGGGTAAAGCCGAGGA CTCTGACAAGACTGCCGTGTAAGGCGC GAGGAAGGTGTGGATGACGT

SEQ ID NO.	Name	Target	DNA Sequence
93	<i>P. denticanis</i> B106 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACC GGCTCT ATGCGATAAGACAGTATGGGGCTAATAG AAATAATTAAGTGAGCCACCTGGGGAGT ACGTCGGCAACGATGAAACTCAAAGGAA TTGACGGGGGCCCGCACAAAGCGGAGGA ACATGTGGTTTAATTCGATGATACGCGAG GAACCTTACCCGGGTTTAAATGTATGTTG CATTATGTAGAAATACGTATTTCTTCGG AACTGCATACAAGGTGCTGCATGGTTGT CGTCAGCTCGTGCCGTGAGGTGTCGGG TTAAGTCCCATAACGAGCGCAACCCTTAT GATTAGTTGCTAACGGTTCAAGCCGAGC ACTCTATTCACACTGCCACCGTAAGGTG CGAGGAAGGAGGGGATGATGTCAAATCA GCACGGCCCTTATATCCGGGGCTACACA CGTGTTACAATGGTCGGTACAGCGGGTT GCATTTACGTGAGTAACAGCTAATCCCAA AAATCGGTCTCAGTTCGGATTGGAGTCT GCAACTCGACTCCATGAAGTTGGATTGCG CTAGTAATCGCACATCAGCCATGGTGCG GTGAATAC
94	<i>P. endodontalis</i> B114 16S rRNA polynucleotide sequence	NA	CACCGCAGTAAACGATGAATACTAGATCT TTGCGATATACGGTAAGGGTCTAAGCGA AAGCGATAAGTATTCCACCTGGGGAGTA CGTCGGCAACGATGAAACTCAAAGGAAT TGACGGGGGCCCGCACAAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAG GAACCTTACCCGGGATTGAAATTTAGCG GGCGGGCTATGAGAGTAGCCTTTCCTAC GGGACTGCTAAGTAGGTGCTGCATGGTT GTCGTCAGCTCGTGCCGTGAGGTGTTGG CTTAAGTGCCATAACGAGCGCAACCCAC GTTGATAGTTACTAACAGTTAAAGCTGAG GACTCTATCGAGACAGCCGGCGTAAGCC GTGAGGAAGGTGTGGATGACGTCAAATC AGCACGGCCCTTACATCCGGGGCGACA CACGTGTTACAATGGTGAGGACAGCGGG AAGCGGCCTGGTGACAGGTAGCAGATCC

SEQ ID NO.	Name	Target	DNA Sequence
			CCAAACCTCATCCCAGTTCGGATTGGAG TCTGCAACTCGACTCTATGAAGCTGGATT CGCTAGTAATCGCGCATCAGCCATGGCG CGGTGAATAC
95	<i>P. gulae</i> B43 <i>fimA</i> polynucleotide sequence	NA	TCTAAATCGAAAAAGATCCTAATAAAACA ATATTCACTTTTAAACAAAAACGAGATG AAAAAGACTAAGTTTTTCTTGTTGGGACT TGCTGCCCTTGCTATGACAGCTTGTAACA AAGACAACGAAGCAGAACCCGTTGTAGA AGGTAACGCTACCATTAGCGTAGTATTGA AGACCAGCAATCCGAATCGTGCTTTCGG GGTTGCAGATGACGAAGCAAAAGTGGCT AAAGTACTGTAATGGTCTACAAGGGTG AGCAGCAGGAAGCCATCAAATCAGCCGA AAATGCAATTAAGGTTGAGAACATCAAAT GTGGTGCAGGCTCACGTACGCTGGTCGT AATGGCCAATACGGGTGGAATGGAATTG GCTGGCAAGACTCTTGCAAGAGGTAAAAG CATTGACAAGTGAAGTAACTGCAGAAAAC CAAGAGGCTACAGGTTTGATCATGACAG CAGAGCCTGTTGACGTAACACTTGTGCG CGGCAATAACTATTATGGTTATGATGGAA CTCAGGGAGGCAATCAGATTCGCAAGG TACTCCTCTTGAAATCAAACGTGTTTCATG CCCGTATTGCGTTCACCAAGATTGAAGT GAAGATGAGCGAGTCTTATGTGAACAAA TACAACCTTACCCCCGAAAACATCTATGC ACTTGTGGCTAAGAAGAAGTCTAATCTAT TCGGTACTTCATTGGCAAATAGTGATGAT GCTTATTTGACCGGTTCTTTGACGACTTT CAACGGTGCTTATACCCCTGCAAACATA CTCATGTCGTCTGGTTGGGAAGAGGCTA CACAGCGCCTTCCAATGATGCTCCACAA GGTTTCTATGTTTTGGAGAGTGCATACGC TCAGAATGCAGGTCTACGTCCTACCATTC



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SEQ ID NO.	Name	Target	DNA Sequence
			TATGTGTAAAGGGTAAGCTGACAAAGCA TGATGGTACTCCTTTGAGTTCTGAGGAAA TGACAGCTGCATTCAATGCCGGCTGGAT TGTTGCAAACAATGATCCTACGACCTATT ATCCTGTATTAGTGAACCTTTGAGAGC
96	<i>P.</i> <i>circumdentari</i> <i>a</i> B52 <i>fimA</i> polynucleotide sequence	NA	TAATGGAGAACAGCAGGAAGCCATCGAA TCAGCCGAAAATGCGACTAAGATTGAGA ATATCAAATGTGGTGCAGGCCAACGTAC GCTGGTCGTAATGGCCAATACGGGTGGA ATGGAATTGGCTGGCAAGACTCTTGCAG AGGTAAAAGCATTGACAAGTGTACTGACT GAAGAAAACCAAGAGGCCACAGGTTTGA TCATGACAGCAGAGCCAAAAGCAATCGT TTTGAAGGCAGGCAAGAACTATATTGGAT ACGATGGAGCCGGAGAGGGCAACCACA TTGAGAATGCTCCTCTTGAAATCAAACGT GTACATGCTCGCATGGCTTTCACCGAAA TTAAAGTACAGATGAGCGCAGCCTACGA TAACATTTACACATTTACCCCTGAAAAGA TTTATGGTCTCATTGCAAAGAAGCAATCT AATTTGTTCTGGGGCAACACTCGTGAATG CAGACGCTAATTATCTGACAGGTTCTTTG ACCACATTTAACGGTGCTTACACACCTAC CAACTATGCCAATGTTCTTGTTGAGCC GTGATTACGTTGCACCTACCGCTGGTGC TCCTCAGGGCTTCTACGTATTAGAAAATG ACTACTCAGCTAACAGTGGAAGTATTCAT CCGACAATCCTGTGTGTTTATGGCAAAC TCAGAAAAACGGAGCCGACCTGACGGGA ACCGATTTAGCAGCAGCTCAGGCCGCCA ATTGGGTGGATGCAGAAGGCAAG

SEQ ID NO.	Name	Target	DNA Sequence
97	<i>P. gulae</i> B69 <i>fimA</i> polynucleotide sequence	NA	GGCGCAGCATAACCTCGACGAACTGCGA CACTATATGCAGGACAATCTCTAAATCGA ATAAAGATTCTAATAAAACAATATTCACCT TTAAACAAAAACAAGATGAAAAAGACTA AGTTTTTCTTGTTGGGACTTGCTGCCCTT GCTATGACAGCTTGTAACAAAGACAACG AAGCAGAACCCGTTGTAGAAGGTAACGC TACCATTAGCGTAGTATTGAAGACCAGCA ATCCGAATCGTGTTTTCGGGGTTGCAGA TGACGAAGCAAAAGTGGCTAAGTTGACC GTAATGGTTTATAATGGAGAACAGCAGG AAGCCATCGAATCAGCCGAAAATGCGAC TAAGATTGAGAATATCAAATGTGGTGCAG GCCAACGTACGCTGGTCGTAAATGGCCAA TACGGGTGGAATGGAATTGGCTGGCAAG ACTCTTGCAGAGGTAAAAGCATTGACAA CTGTACTGACTGAAGAAAACCAAGGGGC CACAGGTTTGATCATGACAGCAGAGCCA AAAGCAATCGTTTTGAAGGCAGGCAAGA ACTATATTGGATACGATGGAGCCGGAGA GGGCAACCACATTGAGAATGCTCCTCTT GAAATCAAACGTGTACATGCTCGCATGG CTTTCACCGAAATTAAAGTACAGATGAGC GCAGCCTACGATAACATTTACACATTTAC CCCTGAAAAGATTTATGGTCTCATTGCAA AGAAGCAATCTAATTTGTTCTGGGGCAAC ACTCGTGAATGCAGACGCTAATTATCTGA CAGGTTCTTTGACCACATTTAACGGTGCT TACACACCTACCAACTATGCCAATGTTCC TTGGTTGAGCCGTGATTACGTTGCACCT ACCGCTGGTGCTCCTCAGGGCTTCTACG TATTAGAAAATGACTACTCAGCTAACAGT GGAACATTATCCGACAATCCTGTGTGT TTATGGCAAACCTTCAGAAAAACGGAGCC GACCTGACGGGAACCGATTTAGCAGCAG CTCAGGCCGCCAATTGGGTGGATGCAGA A

SEQ ID NO.	Name	Target	DNA Sequence
98	<i>P. circumdentaria</i> B97 <i>fimA</i> polynucleotide sequence	NA	TAATGGAGAACAGCAGGAAGCCATCGAA TCAGCCGAAAATGCGACTAAGATTGAGA ATATCAAATGTGGTGCAGGCCAACGTAC GCTGGTCGTAATGGCCAATACGGGTGGA ATGGAATTGGCTGGCAAGACTCTTGCAG AGGTAAAAGCATTGACAACGTACTGACT GAAGAAAACCAAGAGGCCACAGGTTTGA TCATGACAGCAGAGCCAAAAGCAATCGT TTTGAAGGCAGGCAAGAACTATATTGGAT ACGATGGAGCCGGAGAGGGCAACCACA TTGAGAATGCTCCTCTTGAAATCAAACGT GTACATGCTCGCATGGCTTTCACCGAAA TTAAAGTACAGATGAGCGCAGCCTACGA TAACATTTACACATTTACCCCTGAAAAGA TTTATGGTCTCATTGCAAAGAAGCAATCT AATTTGTTGCGGGCAACACTCGTGAATG CAGACGCTAATTATCTGACAGGTTCTTTG ACCACATTTAACGGTGCTTACACACCTAC CAACTATGCCAATGTTCTTGTTGAGCC GTGATTACGTTGCACCTACCGCTGGTGC TCCTCAGGGCTTCTACGTATTAGAAAATG ACTACTCAGCTAACAGTGGAACATTCAT CCGACAATCCTGTGTGTTTATGGCAAAC TCAGAAAAACGGAGCCGACCTGACGGGA ACCGATTTAGCAGCAGCTCAGGCCGCCA ATTGGGTGGATGCAGAAGGCAAG
99	<i>P. cangingivalis</i> B98 <i>fimA</i> polynucleotide sequence	NA	ggcctcgagAACAAAGACAACGAAGCAGAAC CCGTTGTAGAAGGTAACGCTACCATTAG CGTAGTATTGAAGACCAGCAATCCGAAT CGTGCTTTCGGGGTTGCAGATGACGAAG CAAAGTGGCTAAACTGACTGTAATGGT CTACAAGGGTGAGCAGCAGGAAGCCATC AAATCAGCCGAAAATGCAATTAAGGTTGA GAACATCAAATGTGGTGCAGGCTCACGT ACGCTGGTCGTAATGGCCAATACGGGTG GAATGGAATTGGCTGGCAAGACTCTTGC AGAGGTAAAAGCATTGACAACGAACTAA CTGCAGAAAACCAAGAGGCTACAGGTTT

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SEQ ID NO.	Name	Target	DNA Sequence
			GATCATGACAGCAGAGCCTGTTGACGTA ACACTTGTGCGCCGGCAATAACTATTATGG TTATGATGGAACCTCAGGGAGGCAATCAG ATTTGCAAGGTACTCCTCTTGAAATCAA ACGTGTTTCATGCCCCGATTGCGTTCACC AAGATTGAAGTGAAGATGAGCGAGTCTT ATGTGAACAAATACAACCTTACCCCCGAA AACATCTATGCACTTGTGGCTAAGAAGAA GTCTAATCTATTTCGGTACTTCATTGGCAA ATAGTGATGATGCTTATTTGACCGGTTCT TTGACGACTTTCAACGGTGCTTATACCCC TGCAAACCTATACTCATGTCGTCTGGTTGG GAAGAGGCTACACAGCGCCTTCCAATGA TGCTCCACAAGGTTTCTATGTTTTGGAGA GTGCATACGCTCAGAATGCAGGTCTACG TCCTACCATTCTATGTGTAAAGGGTAAGC TGACAAAGCATGATGGTACTCCTTTGAGT TCTGAGGAAATGACAGCTGCATTCAATG CCGGCTGGATTGTTGCAAACAATGATCC TACGACCTATTATCCTGTATTAGTGAAC TTGAGAGCAATAATTACACCTACACAGGT GATGCTGTTGAGAAAGGGAAAATCGTTC GTAACCACAAGTTTGACATCAATCTGACG ATCACCGGTCCTGGTACGAATAATC
100	<i>P. salivosa</i> B104 <i>fimA</i> polynucleotide sequence	NA	TGGCTAARTTGACTGTAATGGTTTATAAT GGAGAACAGCAGGAAGCCATCRAATCAG CCGAAAATGCGACTAAGRRTTGARRAYAT CAAATGTRGTGCAGGCCAACGTACGCTG GTCGTAATGGCCAATACGGGTGSAATGG AAYTGGYTGGCAAGACTCTTGACAGAGGT AAAAGCATTGACAACCTGWACTGACTGMA GAAAACCAAGAGGCYRCAGGKTTGATCA TGACAGCAGAGCCAAAARCAATCGTTTT GAAGGCAGGCAAGAACTAYATTGGATAC RRTGGARCCGGAGAGGGYAAACACATTG AGAATGMTCTCTTTRARATCAARCGTGT WCATGCTCGCATGGCTTTCACCGAAATT AAAGTRCARATGAGCGCAGCCTACGATA

SEQ ID NO.	Name	Target	DNA Sequence
			ACATTTACACATTYRYCCCTGAAAAGATT TATGGTCTCATTGCAAAGAAGCAATCTAA TTTGTTCTGGGGCAACACTCGTRAATGCA GACGCTAATTATCTGACAGGTTCTTTGAC CACATTTAACGGTGCTTACACACCTRCCA ACTATGCCAATGTCCTTGGYTGAGCCG TRATTACGTTGCACCTRCCGCGYGRGTGCT CCTCAGGGYTTCTACGTATTAGAAAATGA CTACTCAGCTAACRGTGGAACATTTCATC CGACAATCCTGTGTGTTTATGGCAAACCT CAGAAAAACGGAGCCGACYTGRCGGGA RCCGATTTAGCARCWGCTCAGGCCGCC AATTGGGTGGATGCAGAAGGCAAGACCT ATTACCCTGTATTTRGTRAACT
101	<i>P. denticanis</i> B106 <i>fimA</i> polynucleotide sequence	NA	TAATGGAGAACAGCAGGAAGCCATCGAA TCAGCCGAAAATGCGACTAAGATTGAGA ATATCAAATGTGGTGACAGGCCAACGTAC GCTGGTCGTAATGGCCAATACGGGTGGA ATGGAATTGGCTGGCAAGACTCTTGACAG AGGTAAAAGCATTGACAACTGTACTGACT GAAGAAAACCAAGAGGCCACAGGTTTGA TCATGACAGCAGAGCCAAAAGCAATCGT TTTGAAGGCAGGCAAGAACTATATTGGAT ACGATGGAGCCGGAGAGGGCAACCACA TTGAGAATGCTCCTCTTGAAATCAAACGT GTACATGCTCGCATGGCTTTCACCGAAA TTAAAGTACAGATGAGCGCAGCCTACGA TAACATTTACACATTTACCCCTGAAAAGA TTTATGGTCTCATTGCAAAGAAGCAATCT AATTTGTTCTGGGGCAACACTCGTGAATG CAGACGCTAATTATCTGACAGGTTCTTTG ACCACATTTAACGGTGCTTACACACCTAC CAACTATGCCAATGTTCTTGGTTGAGCC GTGATTACGTTGCACCTACCGCTGGTGC TCCTCAGGGCTTCTACGTATTAGAAAATG ACTACTCAGCTAACAGTGGAACATTTCAT CCGACAATCCTGTGTGTTTATGGCAAACCT TCAGAAAAACGGAGCCGACCTGACGGGA

SEQ ID NO.	Name	Target	DNA Sequence
			ACCGATTTAGCAGCAGCTCAGGCCGCCA ATTGGGTGGATGCAGAAGGCAAG
102	<i>P. endodontalis</i> B114 <i>fimA</i> polynucleotide sequence	NA	CAAGGGTGAGCAGCAGGAAGCCATCAA TCAGCCGAAAATGCAATTAAGGTTGAGA ACATCAAATGTGGTGCAGGCTCACGTAC GCTGGTCGTAATGGCCAATACGGGTGGA ATGGAATTGGCTGGCAAGACTCTTGACG AGGTAAAAGCATTGACAACTGAACTAACT GCAGAAAACCAAGAGGCTACAGGTTTGA TCATGACAGCAGAGCCTGTTGACGTAAC ACTTGTCGCCGGCAATAACTATTATGGTT ATGATGGAACCTCAGGGAGGCAATCAGAT TTCGCAAGGTACTCCTCTTGAAATCAAAC GTGTTTCATGCCCGTATTGCGTTCACCAA GATTGAAGTGAAGATGAGCGAGTCTTAT GTGAACAAATACAACCTTACCCCCGAAAA CATCTATGCACTTGTGGCTAAGAAGAAGT CTAATCTATTCCGGTACTTCATTGGCAAAT AGTGATGATGCTTATTTGACCGGTTCTTT GACGACTTTCAACGGTGCTTATACCCCT GCAAACCTATACTCATGTCGTCTGGTTGG GAAGAGGCTACACAGCGCCTTCCAATGA TGCTCCACAAGGTTTCTATGTTTTGGAGA GTGCATACGCTCAGAATGCAGGTCTACG TCCTACCATTCTATGTGTAAAGGGTAAGC TGACAAAGCATGATGGTACTCCTTTGAGT TCTGAGGAAATGACAGCTGCATTCAATG CCGGCTGGATTGTTGCAACAATGATCC TACG

SEQ ID NO.	Name	Target	DNA Sequence
103	<i>P. gulae</i> B43 FimA polypeptide sequence	NA	MKKTFFLLGLAALAMTACNKDNEAEPVV EGNATISVVLKTSNPNRAFGVADDEAKVAK LTMVYKGEQQEAIKSAENAIKVENIKCGA GSRTLVMANTGGMELAGKTLAEVKALTT ELTAENQEATGLIMTAEPVDVTLVAGNNYY GYDGTQGGNQISQGTPLIKRVHARIAFTKI EVKMSSESYVNKYNFTPENIYALVAKKSNL FGTSLANSDDAYLTGSLTTFNGAYTPANYT HVVWLGRGYTAPSNDA PQGFVLESAYA QNAGLRPTILCVKGKLT KHDGTPLSSEEMT AAFNAGWIVANNDPTTYYPVLVNFESNNY TYTGDAVEKKGKIVRNHFKFDINLTITGPGTNN PENPITESANLNVNCVVA AWKGVVQNVIV
104	<i>P. circumdentaria</i> B52 FimA polypeptide sequence	NA	NGEQQEAIESAENATK IENIKCGAGQRTL VMANTGGMELAGKTLAEVKALTTVLTEEN QEATGLIMTAEPKAI VLKAGKNYIGYDGAG EGNH IENAPLEIKRVHARMAFTEIKVQMSA AYDNIYFTPEKIYGLIAKKQSNLFGATLVN ADANYLTGSLTTFNGAYTPTNYANVPWLS RDYVAPTAGAPQGFVLENDYSANS GTIH PTILCVYGKLQKNGADLTGTDLAAAQAAN WVDAEG
105	<i>P. gulae</i> B69 FimA AA	NA	MKKTFFLLGLAALAMTACNKDNEAEPVV EGNATISVVLKTSNPNRVFGVADDEAKVAK LTMVYNGEQQEAIESAENATK IENIKCGA GQRTLVMANTGGMELAGKTLAEVKALTT VLTEENQGATGLIMTAEPKAI VLKAGKNYIG YDGAGEGNH IENAPLEIKRVHARMAFTEIK VQMSAAYDNIYFTPEKIYGLIAKKQSNLFG ATLVNADANYLTGSLTTFNGAYTPTNYANV PWLSRDYVAPTAGAPQGFVLENDYSANS GTIHPTILCVYGKLQKNGADLTGTDLAAAQ AANWVDAEGKTYYPVLVNFNSNNYTYDN GYTPKNKIERNH KYDIKLTITGPGTNNPENP ITSAHLNVQCTVAEWVLVGQNATW

SEQ ID NO.	Name	Target	DNA Sequence
106	<i>P. circumdentaria</i> B97 FimA polypeptide sequence	NA	NGEQQEAIESAENATKIENIKCGAGQRTL VMANTGGMELAGKTLAEVKALTTVLTEEN QEATGLIMTAEPKAIVLKAGKNYIGYDGAG EGNHIEAPLEIKRVHARMAFTEIKVQMSA AYDNIYFTPEKIYGLIAKKQSNLFGATLVN ADANYLTGSLTTFNGAYTPTNYANVPWLS RDYVAPTAPQAGFYVLENDYSANSCTIH PTILCVYGLQKNGADLTGTDLAAAQAAN WVDAEG
107	<i>P. cangingivalis</i> B98 FimA AA	NA	VVEGNATISVVLKTSNPNRAFGVADDEAKV AKLTVMVYKGEQQAISAKSAENAIKVENIKC GAGSRTLVMANTGGMELAGKTLAEVKAL TTELTAENQEATGLIMTAEPVDVTLVAGNN YYGYDGTQGGNQISQGTPEIKRVHARIAF TKIEVKMSSEYVNKNFTPENIYALVAKKKS NLFGTSLANSDDAYLTGSLTTFNGAYTPAN YTHVWLGRGYTAPSNAPQAGFYVLESAY AQNAGLRPTILCVKGLTKHDGTPLSSEEM TAAFNAGWIVANNDPTTYYPVLVNFESNN YTYTGDAVEKGKIVRNHFKFDINLTITGPGTN NPENPITESANLNVNCVVAAWK
108	<i>P. salivosa</i> B104 FimA polypeptide sequence	NA	AXLTVMVYNGEQQAISAKSAENATKXXXIKC XAGQRTLVMANTGXMEXXGKTLAEVKAL TTXLTXENQEAXGLIMTAEPKXIVLKAGKNX IGYXGXGEGXHIEAXPLXIXRVHARMAFTEI KVXMSAAYDNIYTXPEKIYGLIAKKQSNLF GATLVNADANYLTGSLTTFNGAYTPXNYA NVPWXSRYVAPXAXAPQAGFYVLENDYSA NXGTIHTILCVYGLQKNGADXXGXDLAX AQAANWVDAEGKTYYPVXVN
109	<i>P. denticanis</i> B106 FimA polypeptide sequence	NA	NGEQQEAIESAENATKIENIKCGAGQRTL VMANTGGMELAGKTLAEVKALTTVLTEEN QEATGLIMTAEPKAIVLKAGKNYIGYDGAG EGNHIEAPLEIKRVHARMAFTEIKVQMSA AYDNIYFTPEKIYGLIAKKQSNLFGATLVN ADANYLTGSLTTFNGAYTPTNYANVPWLS RDYVAPTAPQAGFYVLENDYSANSCTIH PTILCVYGLQKNGADLTGTDLAAAQAAN



SEQ ID NO.	Name	Target	DNA Sequence
			WVDAEG
110	<i>P. endodontalis</i> B114 FimA polypeptide sequence	NA	KGEQQEAIKSAENAIKVENIKCGAGSRTL VMANTGGMELAGKTLAEVKALTTELT AEEN QEATGLIMTAEPVDVTLVAGNNYYGYDGT QGGNQISQGTPLKRVHARIAFTKIEVKMS ESYVNKYNFTPENIYALVAKKSNLFGTSL ANSDDAYLTGSLTTFNGAYTPANYTHVW LGRGYTAPSNDA PQGFVLESAYAQNAGL RPTILCVKGKLT KHDGTPLSSEEMTA AFNA GWIVANNDPT
111	<i>P. gulae</i> B43 <i>oprF</i> polynucleotide sequence	NA	ACATTCGTTGGAGCTATTGCACTGAATGC AAGTGACAGGAAAATACTGTACCGGCA ACGGGTCAGTTACCCGCCAAAAATGTTG CTTTCGCTCGCAACAAAGCAGGCAGCAA TTGGTTCGTAACACTGCAGGGCGGTGTT GCAGCGCAGTTCCTCAATGACAACAACA ACAAAGATTTTGTAGACCGCTTGGGTGC TGCCGGCTCTATTTCAAGTTGGAAAATATC ACAATCCATTCTTTGCAACCCGTTTGCAA ATTAACGGAGCTCAGGCACACACGTTCC TTGGAAAAAATGCGGAACAAGAAATTAAG ACCAATTTTGGCGCAGCTCACTTTGACTT CATGTTTCGATGTGGTTAATTACTTTGCGC CATATCGCGAAAATCGTTTCTTCCATTTA ATTCCATGGGTAGGTGTTGGTTACCAGC ATAAATTCATTGGCAGCAAATGGAGTAAA GACAATGTCGAGTCTCTGACTGCCAATC TGGGTGTTATGATGGCTTTCAGATTAGGA AAACGTGTAGACTTTGTGATCGAAGCAC AAGCAGCACACTCCAATCTCAACTTAAGC CGTGCTTTCAATGCCAAGCCGACTCCTA TTTTCCAGGATCAGGAAGGACGTTATTAC AATGGATTCCAAGGAATGGCGACAGCAG GTCTTAACCTCCGCTTGGGTGCTGTAGG CTTCAATGCCATCGAGCCCATGGACTAC GCGCTTATCAACGATCTGAATGGTCAGA

SEQ ID NO.	Name	Target	DNA Sequence
			TTAATCGCCTGCGCAGAGAAGTCGAAGA ACTCTCCAAGCGTCCTGTATCATGTCCC GAATGCCCCGACGTTACACCCGTTACCA AGACAGAAAACAAGCTAACCGAGAAGGC TGTACTCTTCCGTTTCGACAGCTATGTTG TAGACAAAGACCAGCTTATCAATCTGTAT GACGTAGCTCAGTTTGTAAAAGAAACCAA CGAGCCGATTACTGTTGTAGGCTATGCT GATCCTACGGGTGACACTCAGTACAACG AAAGATTGTCTGAGCGTCGCGCAAAAGC CG
112	<i>P. cansulci</i> B46 <i>oprF</i> polynucleotide sequence	NA	ACATTGGCCGGGGTTTACGCCCTTTCAG CCTCTGCTCAGCAGGAGAATATGCCACG AATGGGGCAGACTCCCGCCAAGAATACC GCTTACGCTCGCTCTGAAGCCGGTGACA ATTGGTTTGTGACTTTGCAAGGAGGTGC TGCTATGCAGTTTGGGAAAGGTAACGAG GATGCCGACTTCTTCGACCGCCAAACTG TTGCTCCCACTTTTGCCGTAGGTAAATGG CACAATCCTTTCTTCGGGACCAGATTGCA AATGGGCTTGGGGGTATCTCACGACTTC TCGAACAACGAAGCGAAATCCAAGTTGG AGATGAACCACGCTCGCTATGCTAACGC ACACTTTGACTTTATGTTTGATGTGATTAA CTACTTCAAGCCCTACAGTGAGGACCGC GTATTCCACCTTATTCCGTGGGTAGGTTT GGGTTACGATCACAAGTTTGAGAAAAAC AGCAACTTCAAGGTGGATGCTCTTACAG CCAACGCCGGTTTGATGTTTGCTTCCGT GTGATGGAGCGTATGGACATTGTGTTGG AAAGCCAGGTAATGTATTCTGACTTCAAC CTCAACACAGCTCTGCCCGAGCCTCGCT ACACAGCTTGCTCCGGCATGCTCACTGC CGGTTTGAAGTTCCGTATAGGAAATATCG GATGGAGCGAGATCCTACCAATGGATTG GGGCTTGGTAAATGACCTGAACGGACAA ATCAACGCCATGCGTGCTAAGAACGCAG

SEQ ID NO.	Name	Target	DNA Sequence
			AGTTGAGCAAGCGTCCCGTTTCTTGCCC CGAATGCCCGGAAGTTGAGCCTCGTGTA GAGCGTATCAATATGCTTTCGGACAAGT CTGTTCTTTTCCGTGCCGGCAAGACAAC TG TAGACAGCGATCAAATGGTAACGATC TTCGACGTAGCTCAGTTTGCAAAGAAGA ATGGCACACAGATCACCGTTACAGGCTA TGCAGACAAGAAGGGCAAAGAAAGCGAT CGCACCTCTGAACTTCGTGCAAAGCCG TAGCCAAGATTCTCACCGACAAGTACGG TGTACCTT
113	<i>P.</i> <i>circumdentari</i> <i>a</i> B52 <i>oprF</i> polynucleotide sequence	NA	TCTATAATGGGAGCTACAGCACTCTCCG CGAGTGCTCAACAATCTACGACACCTGA GACTCAAACCTTTGCCAGCTCGCAAGACG GCTTTTGACCGTTCCGCGGGTCACTGGT TCTTGACTCTACAGGGTGGTGTAATGC ACAGTTTTTGGAGAAAACGAGTCTCAAG ACATCGTAAATCGTCTCCGTGTGATGCC AACTCTTTCTTTAGGAAAGTGGCACAATC CCTATTTTGCAACCCGTTTGCAAGTTTTT GGGGGGCCAACCCCTACTTACTACAAGG AGGTTTCTGGGGAGGTTAAGACCCATAA TACCGCCATGGCTGGAGCTCACTTTGAT TTTATGTTTGATGTAGTAACTTCTATGCA AAGTATAATCCTAAACGAGTATTCCATTT GATTCCTTGGTTCGGTGTGGGATATGGT TTCAAATACTATAACGATTTTGCTGATTTA GCTGATATGATTCAGTTTAATGAACCCTT CCGTCACTCAGCAACTGCGAATGCTGGT TTGATGATGAGTTTTCGCTTGGCAAACG TTTGGATTTGGTTCTGGAAGGGCAGGCT ATATATTCTAACTTGAATATTGTAAAGCAA GAGATAGATTATAAAGCCCCATTATGCC CTATTCAAATATCTACAACGGATTGACAG GTGTCGTTACTGCAGGTCTCAACTTTAAT CTCGGTCGTGTTGCTTGGGAGTCCGTAA CTCCTATGGATATGGATCTTATTAATGAC

SEQ ID NO.	Name	Target	DNA Sequence
			CTAAACGGACAAATTAACCGTTTGCGTTC TGAGAATACAGAGTTGAGAAAACGTCCA GTTTCTTGCCCAGAATGTCCTGAAGTTAC TGCAGAGACGGAAGTAGTTACTGAAAAC GTTTTAGGTGATAAGGCGATTGTTTTCAA GTTTAATAGCGCAACTATTGACAAAGATC AACACATTGTTTTGCAGGATATCGCTGAC TTTGTTAAAGATGGCAACAAAGCTATTGT TGTAATAGGCTTCGCAGATACAACAGGT GATATTAATTACAATATGCATT
114	<i>P. gulae</i> B69 <i>oprF</i> polynucleotide sequence	NA	ACATTCGTTGGAGCTATTGCACTGAATGC AAGTGCACAGGAAAATACTGTACCGGCA ACGGGTCAGTTACCCGCCAAAAATGTTG CTTTTGCCCGCAATAAAGCAGGCGGCAA TTGGTTTGTAACTGCAAGGTGGTGT GCAGCACAGTTCCTTAATGACAACAACAA CAAAGATCTAGTAGACCGCTTAGGAGCT ACCGGATCTATCTCCGTTGGAAAATATCA CAATCCATTCTTTGCGACTCGTTTGCAA TTAACGGAGGTCAAGCACACACGTTCT TGGGAAGAATGCGGAACAAGAAATTAAC ACCAATTTTGGAGCAGCTCACTTTGACTT CATGTTTCGATGTGGTAACTACTTTGCGC CATATCGCGAAAACCGTTTCTTCATTTA ATTCCATGGGTAGGTGTTGGTTACCAAC ACAAATTCATCGGTAGCGAATGGAGTAA AGACAACGTCGAGTCGCTGACCGCAAAC ATGGGTGTTATGATGGCTTTCAGATTAGG GAAGCGCGTGGACTTTGTGATCGAAGCA CAAGCTGCTCACTCCAATCTTAATTTAAG TCGCGCATTCAATGCCAAGAAAACCTCTA TTTCCACGATCAAGAAGGTCGCTATTAC AATGGATTCCAAGGAATGGCTACAGCGG GTCTTAACTTCGCTTAGGTGCTGTTGG CTTCAATGCCATCGAGCCAATGGACTAC GCGCTTATCAACGATCTGAATGGTCAGA

SEQ ID NO.	Name	Target	DNA Sequence
			TTAACCGTTTGCGCAGAGAAGTTGAAGA GCTCTCTAAGCGTCCTGTATCATGCCCC GAATGTCCCGATGTAACACCCGTTACTAA GACAGAAAACAAGCTAACCGAGAAGGCT GTACTCTTCCGCTTCGACAGCTATGTTGT AGACAAAGACCAGCTGATCAATCTGTAT GACGTTGCTCAGTTCGTAAGAAACTAA CGAACCGATTACCGTTGTAGGTTATGCC GATCCTACGGGCAGCACTCAGTACAACG AAAGATTGTCTGAGCGTCGCGCAAAGC CG
115	<i>P.</i> <i>circumdentari</i> <i>a</i> B97 <i>oprF</i> polynucleotide sequence	NA	TCTGTTATGGGAGCTACAGCACTCACAG TTAGTGCTCAGCAACCTACTACACCTGA GACTCAGACATTGCCTGCTCATAAGACG GCTTTTGACCGTTCTGCAGGACATTGGTT CTTGACTCTCCAAGGTGGAGTTAGTGCT CAATTTTTAGAAGAAAATGAAAGTCAAGA AATCTTGAATCGTCTTCATGTTATGCCTA CAATCTCTTTAGGCAAGTGGCACAATCCT TATTTTGCAACTCGTTTGCAAGTGTTTCGG AGGTCCTACTCCTACTTTTTATAAGAATG CTGCTGGTAAGGTGATGAAGGAAAATGC GGCTATGGCTGGGGCTCACTTTGACTTT ATGTTTGATGTTGTGAAGTACTTTGGTAA GTATAATCCAAAGAGAGTCTTTCATCTTG TGCCTTGGTTCGGTGTTGGATATGGCTTT AAATACCATAATGATTCGCCGAAATGAG TGATATCATTAAAGTTTAATGAGCCTTATC GCCATTGAGCAACAGCGAATGCAGGGTT GATGATGAGTTTCCGCTTAGCAAAACGT CTTGATTTAGTGCTTGAAGGACAGGCTAT ATATTCTAATTTGAATATTGTTAAGCAAGA AATTGATTATAAAGCTCCTTCTACTCCTTA TTCTCCAAATTATAATGGGCTTTTGGGAG TTGTTACAGCAGGTCTTAACTTTAATCTT GGTCGTGTTGCTTGGGAGACTGTTACTC CCATGGATATGGATTTGATTAATGATCTT

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SEQ ID NO.	Name	Target	DNA Sequence
			AATGGTCAAATCAATCGTTTGC GTTCTGA GAATACTGAGTTGAGAAAACGTCCTGTTT CTTGTCCTGAATGCCCAGAAGTTTCTAAA GAAACAAC TGTAGTTACAGAAAATGTATT GGGAGACAAAGCTATTGTTTTCAAATTTA ATAGTGCAACTATCAGCAAAGATCAACAT ATTGTTTTGCAAGACATTGCGGACTTTGT TAAGAATGGAAATAAGGGGGTTGCCGTG ATAGGTTTCGCAGATGTAACAGGAGATG CCAATTACAATATGCAAC
116	<i>P.</i> <i>cangingivalis</i> B98 <i>oprF</i> polynucleotide sequence	NA	GGTGGAGTTAGTGCTCAATTTTTAGAAGA AAATGAAAGTCAAGAAATCTTGAATCGTC TTCATGTTATGCCTACAATCTCTTTAGGC AAGTGGCACAATCCTTATTTTGCAACTCG TTTGCAAGTGTTGCGAGGTCCTACTCCTA CTTTTTATAAGAATGCTGCTGGTAAGGTG ATGAAGGAAAATGCGGCTATGGCTGGGG CTCACTTTGACTTTATGTTTGATGTTGTG AACTACTTTGGTAAGTATAATCCAAAGAG AGTCTTTCATCTTGTGCCTTGGTTCGGTG TTGGATATGGCTTTAAATACCATAATGAT TTCGCCGAAATGAGTGATATCATTAAAGTT TAATGAGCCTTATCGCCATTGAGCAACAG CGAATGCAGGGTTGATGATGAGTTTCCG CTTAGCAAAACGTCCTTGATTTAGTGCTTG AAGGACAGGCTATATATTCTAATTTGAAT ATTGTTAAGCAAGAAATTGATTATAAAGC TCCTTCTACTCCTTATTCTCAAATTATAA TGGGCTTTTGGGAGTTGTTACAGCAGGT CTTAAC TTTAATCTTGGTCGTGTTGCTTG GGAGACTGTTACTCCCATGGATATGGAT TTGATTAATGATCTTAATGGTCAAATCAAT CGTTTGCGTTCTGAGAATACTGAGTTGA GAAAACGTCCTGTTTCTTGTCCTGAATGC CCAGAAGTTTCTAAAGAAACAAC TGTAGT TACAGAAAATGTATTGGGAGACAAAGCTA

SEQ ID NO.	Name	Target	DNA Sequence
			TTGTTTTCAAATTTAATAGTGCAACTATCA GCAAAGATCAACATATTGTTTTGCAAGAC ATTGCGGACTTTGTTAAGAATGGAAATAA GGGGGTTGCCGTGATAGGTTTCGCAGAT GTAACAGGAGATGCCAATTACAATATGCA ACTTTCTGAACGTCGTGCTAAGGCTGTT GCGGAAGCTCTTGTGAATCAATTC
117	<i>P. salivosa</i> B104 <i>oprF</i> polynucleotide sequence	NA	CATTGGTTCTTGACTCTCCAAGGTGGAG TTAGTGCTCAATTTTTAGAAGAAAATGAA AGTCAAGAAATCTTGAATCGTCTTCATGT TATGCCTACAATCTCTTTAGGCAAGTGGC ACAATCCTTATTTTGCAACTCGTTTGCAA GTGTTCCGGAGGTCCTACTCCTACTTTTTA TAAGAATGCTGCTGGTAAGGTGATGAAG GAAAATGCGGCTATGGCTGGGGCTCACT TTGACTTTATGTTTGATGTTGTGAACTAC TTTGGTAAGTATAATCCAAAGAGAGTCTT TCATCTTGTGCCTTGGTTCGGTGTGATG ATGGCTTTAAATACCATAATGATTTCGCC GAAATGAGTGATATCATTAAAGTTTAATGA GCCTTATCGCCATTGAGCAACAGCGAAT GCAGGGTTGATGATGAGTTTCCGCTTAG CAAAACGTCTTGATTTAGTGCTTGAAGGA CAGGCTATATATTCTAATTTGAATATTGTT AAGCAAGAAATTGATTATAAAGCTCCTTC TACTCCTTATTCTCAAATTATAATGGGC TTTTGGGAGTTGTTACAGCAGGTCTTAAC TTTAATCTTGGTCGTGTTGCCTGGGAGA CTATTACTCCCATGGATATGGATTTGATT AATGATCTTAATGGTCAAATCAATCGTTT GCGTTCTGAGAATACTGAGTTGAGAAAA CGTCCTGTTTCTTGTCTGAATGCCCAGA AGTTTCTAAAGAAACAACGTAGTTACAG AAAATGTATTGGGAGACAAAGCTATTGTT

SEQ ID NO.	Name	Target	DNA Sequence
			TTCAAATTTAATAGTGCAACTATCAGCAA AGATCAACATATTGTTTTGCAAGACATTG CGGACTTTGTTAAGAATGGAAATAAGGG GGTTGCCGTGATAGGTTTCGCAGATGTA ACAGGAGATGCCAATTACAATATGCAACT TTCTGAACGTCGTGCTAAGGCTGTTGCG GAAGCTCTTGTGAATCAATTC
118	<i>P. denticanis</i> B106 <i>oprF</i> polynucleotide sequence	NA	GCTCATAAGACGGCTTTTGACCGTTCTG CAGGACATTGGTTCTTGACTCTCCAAGG TGGAGTTAGTGCTCAATTTTAGAAGAAA ATGAAAGTCAAGAAATCTTGAATCGTCTT CATGTTATGCCTACAATCTCTTTAGGCAA GTGGCACAATCCTTATTTTGCAACTCGTT TGCAAGTGTTCCGAGGTCCTACTCCTAC TTTTATAAGAATGCTGCTGGTAAGGTGA TGAAGGAAAATGCGGCTATGGCTGGGGC TCACTTTGACTTTATGTTTGATGTTGTGA ACTACTTTGGTAAGTATAATCCAAAGAGA GTCTTTCATCTTGTGCCTTGGTTCGGTGT TGGATATGGCTTTAAATACCATAATGATT TCGCCGAAATGAGTGATATCATTAAGTTT AATGAGCCTTATCGCCATTCAGCAACAG CGAATGCAGGGTTGATGATGAGTTCCG CTTAGCAAAACGTCTTGATTTAGTGCTTG AAGGACAGGCTATATATTCTAATTTGAAT ATTGTTAAGCAAGAAATTGATTATAAAGC TCCTTCTACTCCTTATTCTCAAATTATAA TGGGCTTTTGGGAGTTGTTACAGCAGGT CTTAACTTTAATCTTGGTCGTGTTGCTTG GGAGACTGTTACTCCCATGGATATGGAT TTGATTAATGATCTTAATGGTCAAATCAAT CGTTTGCGTTCTGAGAATACTGAGTTGA GAAACGTCCTGTTTCTTGTCTGAATGC



SEQ ID NO.	Name	Target	DNA Sequence
			CCAGAAGTTTCTAAAGAAACAACACTGTAGT TACAGAAAATGTATTGGGAGACAAAGCTA TTGTTTTCAAATTTAATAGTGCAACTATCA GCAAAGATCAACATATTGTTTTGCAAGAC ATTGCGGACTTTGTGAAGAATGGAAATAA GGGGGTTGCCGTGATAGGTTTCGCAGAT GTAACAGGAGATGCCAATTACAATATGCA ACTTTCTGAACGTCGTGCTAAGGCTGTT GCGGAAGCTCTTGTGAATCAATTCGGAG TTCCTTCTGATATGATT
119	<i>P.</i> <i>endodontalis</i> B114 <i>oprF</i> polynucleotide sequence	NA	TCAGCACTGGGGGCTTTGGCACTTACAG CTAGTGCTCAACAACTACGAAACCAGC GAATAGTATGCCCGCATTCAAGACTGCA TTTGAACGCAGCGGCGGTCAATTGGTTTC TGACAATTCAGGGTGGCCTGAGTGCTCA ACTTTTGGGTGAAAATGAAAAGATGGACT TTGGCAAGCGTCTGCTACATGCTGCCAA GGCCAGTGACAACACCCAAACAGAGGCT AGCTACCTACGCATCATGCCACGCTCT CTGTAGGTAAATGGCATAATCCCTACTTT GCTACTCGTGTACAGCTCTTCGGTGGTC TCACTCCTCTCTACAATACTGAGGGTGG CGTTAATGTACACACCTACAACACTGCCA CGATCGGTGCCCACTATGATTTCATGTTT GATGTAGTAACTATTTGCGCAAGTACAA CCCCAAACGTTTCTTCCACGTAATTCCTT GGGTGGGTCTTGGTTACAACCTCAAGTA TCATGATGTATTTGGATTCAAGGAGCCCT ATCGTCACTCTGTACAGGTAACGCAGG CATGGAGTTTGCTTTCCGCCTCGGTAAG CGTGTAGACCTTGTAAGCTCGAAGCTCAGG TAGTGTACAACAACCTGAACCTGATCAAG CAGGAAGTCGACTACGATGTAGTCACTA CTCCCTATGTACCTGCTGATACATACGCT GGTCTTATGACCATGTTTACTGCTGGTCT TAACTTCAATCTGGGCAAGGTTGAGTGG

SEQ ID NO.	Name	Target	DNA Sequence
			GAAACTGTTGAGCCGATGGACTACCAGC TCATAAACGACTTGAAGTCTCAGATCAGC CGTCTACGTAGCGAAAACGCAGAGCTTT CCAAGCGTCCTGCTTTCTGCCCCGAGTG TCCCGAAGTAGAGGAAGTAGAAGATGTT GTTGTTGACCAGTATGTCCTCACCGACA AGGCTATCCTCTTCGACTTTGACAAGAG CAACATCCGCAAGGACCAACAAGCTCAG CTTGGTATGATTGCTGAATTCGTGAAGAA GTACAATACGCCTATCGTGGTAGTAGGC TATG
120	<i>P. gulae</i> B43 OprF polypeptide sequence	NA	TFVGAIALNASAQENTVPATGQLPAKNVAF ARNKAGSNWFVTLQGGVAAQFLNDNNNK DFVDRLGAAGSISVGKYHNPFFATRLQING AQAHFTFLGKNAEQEIKTNFGAAHFDFMFD VVNYFAPYRENRRFFHLIPWVGVGQYHKFIG SKWSKDNVESLTANLGVMMAFRLGKRVD FVIEAQAHSNLNLSRAFNAPKPTPIFQDQE GRYYNGFQGMATAGLNFRLGAVGFNAIEP MDYALINDLNGQINRLRREVEELSKRPVSC PECPDVTPTKTENKLTEKAVLFRFDSYVV DKDQLINLYDVAQFVKETNEPITVVGADP TGDTQYNERLSERRAKAVVDVLTGKYGVP SELISVEWKGDTTQPFNKKAWN
121	<i>P. cansulci</i> B46 OprF polypeptide sequence	NA	TLAGVYALSASAQQENMPRMGQTPAKNT AYARSEAGDNWFVTLQGGGAAMQFGKGNE DADFFDRQTVAPTFVAGKWHNPFFGTRLQ MGLGVSHDFSNNNAKSKLEMNHARYANA HDFMFDVINYFKPYSEDRVFHLIPWVGLG YDHKFEKNSNFKVDALTANAGLMFAFRVM ERMDIVLESQVMYSDFNLNTALPEPRYTAC SGMLTAGLNFRIGNIGWSEILPMDWGLVN DLNGQINAMRAKNAELSKRPVSCPECEV EPRVERINMLSDKSVLFRAGKTTVDSQDM VTIFDVAQFAKNGTQITVTGYADKKGKES DRTSELRAKAVAKILTDKYGVPSDRISIEWK GVSEQVVDNRDWNRRV

SEQ ID NO.	Name	Target	DNA Sequence
122	<i>P. circumdentaria</i> B52 OprF polypeptide sequence	NA	SIMGATALSASAQQSTTPETQTLPAKRTAF DRSAGHWFLTLQGGVNAQFLEENESQDIV NRLRVMPTLSLGKWHNPYFATRLQVFGGP TPTYKEVSGEVKLTNTAMAGAHFDFMFD VVNFYAKYNPKRVFHLIPWFGVGYGFKYY NDFADLADMIQFNEPFRHSATANAGLMMS FRLAKRLDLVLEGQAIYSNLNIVKQEIDYKA PIMPYSNIYNGLTGVVTAGLNFNLGRVAWE SVTPMDMDLINDLNGQINRLRSENTELKRK PVSCPECPEVTAETEVVTENVLGDKAIVFK FNSATIDKDQHIVLQDIADFVKDGNKAIVVI GFADTTGDINYNMHLSEERRAKAVAEALVN KFGVSSDMISVEWQGETEQFNPAWN
123	<i>P. gulyae</i> B69 OprF polypeptide sequence	NA	TFVGAIALNASAQENTVPATGQLPAKNVAF ARNKAGGNWFVTLQGGVAAQFLNDNNNK DLVDRLGATGSISVGKYHNPFFATRLQING GQAHTFLGKNAEQEINTNFGAAHFDFMFD VVNYFAPYRENRRFFHLIPWVGVGQYHKFIG SEWSKDNVESLTANMGVMMAFRLGKRVD FVIEAQAHSNLNLSRAFNAKKTPIFHDQE GRYYNGFQGMATAGLNFRLGAVGFNAIEP MDYALINDLNGQINRLRREVEELSKRPVSC PECPDVTPVTKTENKLTEKAVLFRFDSYVV DKDQLINLYDVAQFVKETNEPITVVGADP TGSTQYNERLSERRAKAVDVLTKGYGVP SELISVEWKGDSTQPFNKKAWN
124	<i>P. circumdentaria</i> B97 OprF polypeptide sequence	NA	SVMGATALTVSAQQPTTPETQTLPAHKTA FDRSAGHWFLTLQGGVSAQFLEENESQEI LNRLHVMPTISLGKWHNPYFATRLQVFGG PTPTFYKNAAGKVMKENAAMAGAHFDFMF DVVNYFGKYNPKRVFHLVPWFGVGYGFK YHNDFAEMSDIKFNEPYRHSATANAGLM MSFRLAKRLDLVLEGQAIYSNLNIVKQEIDY KAPSTPYSNPNYNGLLGVVTAGLNFNLGRV AWETVTPMDMDLINDLNGQINRLRSENTEL RKRPVSCPECPEVSKETTVVTENVLGDKAI VFKFNSATISKDQHIVLQDIADFVKNGNKG VAVIGFADVTGDANYNMLSEERRAKAVAE

SEQ ID NO.	Name	Target	DNA Sequence
			ALVNQFGVPSDMISVEWQGETELFEARAW N
125	<i>P. cangingivalis</i> B98 OprF polypeptide sequence	NA	GGVSAQFLEENESQEILNRLHVMPPTISLGK WHNPYFATRLQVFGGPTPTFYKNAAGKV MKENAAMAGAHFDFMFDVVNYFGKYNPK RVFHLVPWFGVGYGFKYHNDFAEMSDIIF NEPYRHSATANAGLMMSFRLAKRLDLVLE GQAIYSNLNIVKQEIDYKAPSTPYSPNYNGL LGVVTAGLNFNLGRVAWETVTPMDMDLIN DLNGQINRLRSENTELKRKPVSCPECPEV SKETTVVTENVLGDKAIVFKFNSATISKDQH IVLQDIADFVKNGNKGVAVIGFADVTGDAN YNMQLSERRAKAVAEALVNQF
126	<i>P. salivosa</i> B104 OprF polypeptide sequence	NA	HWFLTQGGVSAQFLEENESQEILNRLHV MPTISLGKWHNPYFATRLQVFGGPTPTFY KNAAGKVMKENAAMAGAHFDFMFDVVNY FGKYNPKRVFHLVPWFGVGYGFKYHNDFA EMSDIIFNEPYRHSATANAGLMMSFRLA KRLDLVLEGQAIYSNLNIVKQEIDYKAPSTP YSPNYNGLLGVVTAGLNFNLGRVAWETITP MDMDLINDLNGQINRLRSENTELKRKPVSC PECPEVSKETTVVTENVLGDKAIVFKFNSA TISKDQHIVLQDIADFVKNGNKGVAVIGFAD VTGDANYNMQLSERRAKAVAEALVNQF
127	<i>P. denticanis</i> B106 OprF polypeptide sequence	NA	AHKTAFDERSAGHWFLTQGGVSAQFLEEN ESQEILNRLHVMPPTISLGKWHNPYFATRLQ VFGGPTPTFYKNAAGKVMKENAAMAGAH FDFMFDVVNYFGKYNPKRVFHLVPWFGV GYGFKYHNDFAEMSDIIFNEPYRHSATAN AGLMMSFRLAKRLDLVLEGQAIYSNLNIVK QEIDYKAPSTPYSPNYNGLLGVVTAGLNFN LGRVAWETVTPMDMDLINDLNGQINRLRS ENTELKRKPVSCPECPEVSKETTVVTENVL GDKAIVFKFNSATISKDQHIVLQDIADFVK NKGVAVIGFADVTGDANYNMQLSERRAK

SEQ ID NO.	Name	Target	DNA Sequence
			AVAEALVNQFGVPSDMISVEWQGET
128	<i>P. endodontalis</i> B114 OprF polypeptide sequence	NA	SALGALALTASAQQTTPANSMPAFKTAFE RSGGHWFLTIQGGLSAQLLGENEKMDFGK RLLHAAKASDNTQTEASYLRIMPTLSVGKW HNPYFATRVQLFGGLTPLYNTEGGVNVHT YNTATIGAHYDFMFDVWNYFAKYNPKRFFH VIPWVGLGYNFKYHDVFGFKEPYRHSVTG NAGMEFAFRLGKRVDLVLEAQVVYNNLNI KQEVLDYDVWTPYVPADTYAGLMTMFTAG LNFNLGKVEWETVEPMDYQLINDLNSQISR LRSENAELSKRPAFCPECPEVEEVEDVV DQYVLTDKAILFDFDKSNIRKDQQAQLGMI AEFVKKYNTPIVVVGADPTGKSKYNMELS KRRQAQAVNELTNRHGVPADLITMEWEGA TNKFTPPTAWN
129	<i>P. gulae</i> B43 FimA polypeptide fragment sequence #1	NA	ACNKDNEAEPVV
130	<i>P. gulae</i> B43 FimA polypeptide fragment sequence #2	NA	YPVLVNFESNNYTYTGDAVEK
131	<i>P. gulae</i> B43 FimA polypeptide fragment sequence #3	NA	TGPGTNNPENPITESA
132	<i>P. gulae</i> B43 OprF polypeptide fragment	NA	NDNNNKDFVDRLGA

SEQ ID NO.	Name	Target	DNA Sequence
	sequence #1		
133	<i>P. gulae</i> B43 OprF polypeptide fragment sequence #2	NA	DLNGQINRLRREVEELSKRPVSCPECPDV
134	<i>P. gulae</i> B43 OprF polypeptide fragment sequence #3	NA	ADPTGDTQYNERLSERRAKAV
135	pBAD-HisA Amino- terminal polypeptide sequence	NA	MGGSHHHHHHGMASMTGGQMGRDLYDD DDKDRWGSELEICSQYHMG
136	pBAD-TOPO Amino- terminal polypeptide sequence	NA	MGSGSGDDDDKLALM
137	I vector Amino- terminal polypeptide sequence	NA	MGTTTTTSLHM

**Note:** Lower case nucleotides are not present in the target DNA sequence. They are added to the 5' region of the primer to aid in cloning. NA, Not applicable

The following companion animal periodontal isolates were deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA, 20110, USA, on August 9, 2001: *P. gulae* B43 (PTA-3618), *P. cansulci* B46 (PTA-3619), *P. circumdentaria* B52 (PTA-3620), *P. gulae* B69 (PTA-3621), *P. circumdentaria* B97 (PTA-3622), *P. cangingivalis* B98 (PTA-3623), *P. salivosa* B104 (PTA-3624), *P. denticanis* B106 (PTA-3625), and *P. endodontalis* B114 (PTA-3626). In a preferred embodiment of the

invention, an isolated polynucleotide molecule of the present invention has a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 102 and 111 to 119. The preferred polypeptides of the present invention have amino acid sequences selected from the group consisting of SEQ ID NOS: 103 to 110 and 120 to 128.

5                                    **Cloning of Porphyromonas Nucleotide Sequences**

There are several known methods or techniques that can be used to clone the *Porphyromonas* nucleotide sequences of the present invention. For example, the sequences can be isolated as restriction fragments and cloned into cloning and/or expression vectors, the sequences can be PCR amplified and cloned into cloning and/or expression vectors, or  
10    the sequences can be cloned by a combination of these two methods.

Standard molecular biology techniques known in the art and not specifically described can be generally followed as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (1989); Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989); Perbal, A  
15    *Practical Guide to Molecular Cloning*, John Wiley & Sons, New York (1988); Watson et al., *Recombinant DNA*, Scientific American Books, New York; Birren et al (eds) *Genome Analysis: A Laboratory Manual Series, Vols. 1-4* Cold Spring Harbor Laboratory Press, New York (1998); and methodology set forth in United States Patent Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057. Polymerase chain reaction (PCR) is carried out  
20    generally as described in *PCR Protocols: A Guide To Methods And Applications*, Academic Press, San Diego, CA (1990).

Examples of methods useful in cloning and sequencing the polynucleotides of the present invention are provided in the Example.

**fimA and oprF-ENCODED POLYPEPTIDES AND PROTEINS**

25                    The present invention encompasses the use of prokaryotic and eukaryotic expression systems, including vectors and host cells, which may be used to express both truncated and full-length (native protein) forms of the recombinant polypeptides expressed by the nucleotide sequences of the present invention.

In a preferred embodiment of the invention, an isolated polynucleotide molecule of  
30    the present invention has a nucleotide sequence selected from one of the sequences of SEQ ID NO:95 to 102 and 111 to 119 or degenerate variants thereof; and encoding a corresponding polypeptide selected from the amino acid sequences of SEQ ID NO:103 to 110 and 120 to 128, respectively.

A variety of host-expression vector systems may be utilized to express the  
35    polypeptides of the present invention. Such host-expression systems also represent vehicles by which the coding sequences of interest may be cloned and subsequently purified. The present invention further provides for host cells which may, when transformed or transfected with the appropriate vector or nucleotide sequence, express the encoded polypeptide gene product of the invention. Such host cells, include but are not limited to, microorganisms such

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as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing the gene product coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In a preferred embodiment, the expression system is a bacterial system. A number of expression vectors may be advantageously selected depending upon the use intended for the product being expressed. For example, when a large quantity of such a polypeptide is to be produced, for the generation of vaccine compositions or for raising antibodies, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Preferably, the vectors contain promoters that direct inducible gene expression. Suitable vectors include, but are not limited to, the *E. coli* pET expression vectors (Studier and Moffatt, 1986, J. Mol. Biol. 189:113; Rosenberg et al., 1987, Gene 56:125-135; Novagen, Madison, Wisconsin), in which the coding sequence can be fused in-frame to a sequence encoding multiple (e.g., 6) histidine residues; pBAD vectors (Guzman et al., 1995, J. Bact. 177:4121-4130), from which a heterologous protein can be expressed under the control of an arabinose inducible protein; and pGEX vectors (Pharmacia Biotech, USA), used to express heterologous polypeptides as fusion proteins with glutathione S-transferase (GST). The *fimA* or *oprF* sequences of the present invention can be cloned into a  $\lambda$  expression vector and expressed in  $\lambda^-$  bacterial strains. In a preferred mode, the bacterial strain is *E. coli* BL21 (Gibco-BRL, USA). Preferably, the vectors that can be used include, but are not limited to, pLEX expression vectors (LaVallie et al., 1992, Bio/Technology 11:187-193; Mieschendahl et al., 1986, Bio/Technology 4:802-808; Invitrogen) and pRIT2T expression vectors (Nilsson et al., 1985, EMBO 4:1075; Zabeau and Stanley, 1982, EMBO 1:1217; Pharmacia Biotech). Other vectors and bacterial strains can be used and are known to those skilled in the art.

#### **Antibody Production**

Antibodies may either be monoclonal, polyclonal, or recombinant. Conveniently, the antibodies may be prepared against the immunogen or portion thereof, for example, a synthetic peptide based on the sequence, or prepared recombinantly by cloning techniques or the natural gene product and/or portions thereof may be isolated and used as the immunogen. Immunogens can be used to produce antibodies by standard antibody production technology well known to those skilled in the art as described generally in Harlow



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and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992. Antibody fragments may also be prepared from the antibodies and include Fab, F(ab')<sub>2</sub>, and Fv by methods known to those skilled in the art.

5 In the production of antibodies, screening for the desired antibody can be accomplished by standard methods in immunology known in the art. Techniques not specifically described are generally followed as in Stites et al.(eds), *Basic and Clinical Immunology* (8th Edition), Appleton & Lange, Norwalk, CT (1994) and Mishell and Shiigi (eds), *Selected Methods in Cellular Immunology*, W.H. Freeman and Co., New York (1980).  
10 In general, ELISAs and Western blotting are the preferred types of immunoassays. Both assays are well known to those skilled in the art. Both polyclonal and monoclonal antibodies can be used in the assays. The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or be both bound and conjugated as is well known in the art (for a general discussion of conjugation of fluorescent or enzymatic moieties see  
15 Johnstone & Thorpe, *Immunochemistry in Practice*, Blackwell Scientific Publications, Oxford, 1982.) The binding of antibodies to a solid support substrate is also well known in the art (see for a general discussion, Harlow & Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Publications, New York, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992). The detectable moieties contemplated for  
20 use in the present invention can include, but are not limited to, fluorescent, metallic, enzymatic and radioactive markers such as biotin, gold, ferritin, alkaline phosphatase, b-galactosidase, peroxidase, urease, fluorescein, rhodamine, tritium, <sup>14</sup>C and iodination.

Where appropriate, other immunoassays such as radioimmunoassays (RIA) can be used as known in the art. Available immunoassays are extensively described in the patent  
25 and scientific literature. See, for example, United States Patent Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771; and 5,281,521, as well as Sambrook et al, *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor, New York, 1989.

#### **Detection, Diagnostic, and Prevention Kits**

30 The present invention further provides kits for the detection of *Porphyromonas* spp. The kit includes reagents for analyzing a sample for the presence of *Porphyromonas* organisms, polypeptides, or *Porphyromonas* nucleotide sequences of the present invention, wherein the presence of the nucleotide sequence is indicative of the presence of the organism. This method is valuable because disease can be diagnosed prior to the existence  
35 of symptoms and can therefore prevent the onset of the disease prior to the occurrence of damage to the patient. The presence of the *Porphyromonas* spp. Bacteria, polypeptides, or nucleotide sequences can be determined using antibodies, PCR, hybridization, and other detection methods known to those of skill in the art.

In one embodiment, the kit provides reagents for the detection of antibodies against *Porphyromonas*. In certain embodiments, the kit can include a set of printed instructions or a label indicating that the kit is useful for the detection of *Porphyromonas* spp. Minimally, the kit comprises in at least one container a protein having an amino acid sequence comprising at least 30 contiguous amino acids of any of the polypeptides of SEQ ID NO:103 to 110 and 120 to 128. In one embodiment, the kit further comprises a secondary antibody. In a preferred embodiment, the secondary antibody is conjugated to a detectable moiety, such as, e.g., an enzyme that catalyzes a colorimetric or chemiluminescent reaction, such as alkaline phosphatase or horseradish peroxidase. In a further embodiment, the kit comprises reagents for carrying out a colorimetric or chemiluminescent assay.

In another embodiment, the kit provides reagents for the detection of *Porphyromonas* nucleic acids. In one embodiment, the kit provides reagents for the PCR detection of *Porphyromonas* nucleic acids and comprises in at least one container a first isolated DNA molecule comprising a fragment of at least about 15, 20, 25 or 30 nucleotides, which fragment hybridizes under stringent conditions to a DNA molecule encoding a polypeptide comprising a sequence of at least 5, 10, 15, 20, 25, or 30 contiguous amino acids, or the complete amino acid sequence, of any of the polypeptides of SEQ ID NO:xx-yy, and a second isolated DNA molecule comprising a fragment of at least 15, 20, 25, or 30 nucleotides, which fragment hybridizes under stringent conditions to a DNA molecule complementary to a DNA molecule encoding a polypeptide having a sequence of at least 5, 10, 15, 20, 25, or 30 contiguous amino acids, or the complete amino acid sequence, of any of the polypeptides of SEQ ID NO:xx-yy, which first and second DNA molecules can be used to specifically amplify a *Porphyromonas* spp. nucleic acid encoding a 16S rRNA which 16S rRNA is encoded by a DNA molecule selected from the group consisting of SEQ ID NOS: 1-9.

In an further embodiment, the present invention provides a kit comprising in at least one container an isolated DNA molecule comprising a nucleotide sequence of at least about 15 contiguous nucleotides selected from any of SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119 which hybridizes under highly stringent conditions to the complement of any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119, and a second isolated DNA molecule comprising in a second container an isolated DNA molecule comprising a nucleotide sequence of at least about 15 contiguous nucleotides selected from the complement of any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119 which hybridizes under highly stringent conditions to any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119, wherein the kit further comprises a set of instructions indicating that the kit is useful for the detection of *Porphyromonas* spp.

#### **Vaccine Formulation and Method of Administration**

The vaccine of the present invention can be administered to a companion animal in an effective amount such that the vaccine therapeutically treats or confers resistance to or

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prevents periodontal disease in the companion animal. The vaccine of the present invention is useful in the control of bacteria that cause periodontal disease. The vaccines of the present invention can, in particular, be used in the field of veterinary medicine to treat companion animals and for the maintenance of public health against those bacteria described herein  
5 which are known to cause periodontal disease.

The vaccines of the present invention are of value in the control of bacteria that are injurious to, or spread or act as vectors of disease in man and companion animals, for example those described herein. The vaccines of the present invention are particularly useful in controlling bacteria that are present in companion animals for which purpose they can be  
10 administered using any known methods of administration, including, but not limited to, oral, parenteral, intranasal, subcutaneous, or topical.

According to a further aspect of the present invention, there is provided a composition comprising a vaccine of the present invention, in admixture with a compatible adjuvant, diluent or carrier. In a preferred embodiment, the vaccine formulation of the present invention  
15 is composed of an aqueous suspension or solution containing at least one bacteria of the present invention and/or at least one subunit protein, preferably buffered at physiological pH, in a form ready for injection.

The present invention further provides a method of treating or preventing a bacterial infection, which comprises treatment with an effective amount of a vaccine or vaccine  
20 formulation of the present invention. It is to be appreciated that reference to treatment includes prophylaxis as well as the alleviation of established symptoms of a bacterial infection.

The vaccines and vaccine formulations of the present invention can be used to induce a response that prevents the pathological changes characteristic of periodontal disease caused  
25 by periodontal disease-causing bacteria. In a vaccine formulation, an immunogenic amount of the bacteria, purified protein, nucleic acid, or combinations thereof is desirably mixed with a suitable conventional vaccine adjuvants and physiologic vehicles, for use in mammals.

A vaccine formulation for preventing periodontal disease in companion animals can be produced using at least one of the isolated and purified inactivated or attenuated bacteria,  
30 purified polypeptides (such as native proteins, subunit proteins, or polypeptides, and admixing one or more of these with a compatible adjuvant, diluent, or carrier. Preferably, the polypeptide sequences are subunit proteins selected from the group including FimA (SEQ ID NOS: 103 to 110 and OprF (SEQ ID NOS: 120 to 128).

Examples of fragments of FimA and OprF that can be used for diagnostic  
35 polypeptides or for vaccine preparations include, but are not limited to ACNKDNEAEPVV, YPVLVNFESNNYTYTGDAVEK, TGPGTNNPENPITESA, NDNNNKDFVDRLGA, DLNGQINRLRREVEELSKRPVSCPECPDV, and ADPTGDTQYNERLSERRAKAV (SEQ ID NOS: 129-134). The subunit protein can be recombinantly expressed, either alone or fused to another polypeptide sequence or protein. The other polypeptide sequence or protein can

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include, but is not limited to, a poly-His tag, MBP, thioredoxin, or GST, for example. Also provided by the present invention are the polynucleotide sequences or genes that encode any of the above mentioned subunit proteins. The polynucleotide sequence of the bacteria can be selected from *fimA* and *oprF* or a fragment or variant thereof which fragment or variant exhibits at least about 90%, 95%, or 99% homology thereto, or a complementary polynucleotide sequence which hybridizes under high stringency conditions, or a combination of both. Preferably, the polynucleotide sequences of the present invention can be used to amplify a *fimA* or *oprF* DNA molecule of the present invention, or encodes an amino acid fragment than can be used to raise antibodies against FimA or OprF.

For DNA-based therapy, a vehicle capable of delivering or transferring heterologous nucleic acid into a host cell may be used. The expression vehicle may include elements to control targeting, expression and transcription of the nucleic acid in a cell selective manner as is known in the art. The expression vehicle can include a promoter for controlling transcription of the heterologous material and can be either a constitutive or inducible promoter to allow selective transcription. Enhancers that may be required to obtain necessary transcription levels can optionally be included.

Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992); Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989); Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, MI (1995); Vega et al., *Gene Targeting*, CRC Press, Ann Arbor, MI (1995); R.L. Rodriguez *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston MA (1988) and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors.

The present invention further provides for combinations vaccines having at least one of the inactivated or attenuated bacteria, nucleotide sequences, or polypeptide sequences of the present invention, in combination with one or more additional immunogenic components. Such a combination vaccine may produce in the vaccinated animal a surprisingly greater effect than that expected by simply adding the effects of each component administered separately. Thus, a combination vaccine may stimulate a synergistic production of antibody in animals.

Vaccines of the present invention can be prepared by combination of at least one of the inactivated or attenuated bacteria, nucleotide sequences, or polypeptide sequences of the present invention, with a pharmaceutically acceptable carrier, an preferably, an adjuvant.

Suitable preparations of the vaccines of the present invention include injectables, either liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, a liquid pharmaceutically acceptable carrier prior to injection may also be prepared. The vaccine preparation may be emulsified. The active immunogenic component, is preferably

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mixed with an adjuvant which is pharmaceutically acceptable and compatible with the active immunogenic component. Suitable adjuvants include, but are not limited to: mineral gels, e.g., aluminum hydroxide; surface active substances such as lysolecithin; glycosides, e.g., saponin derivatives such as Quil A or GPI-0100 (United States Patent No. 5,977,081); cationic surfactants such as DDA, pluronic polyols; polyanions; non-ionic block polymers, e.g., Pluronic F-127 (B.A.S.F., USA); peptides; mineral oils, e.g. Montanide ISA-50 (Seppic, Paris, France), carbopol, Amphigen (Hydronics, Omaha, NE USA), Alhydrogel (Superfos Biosector, Frederikssund, Denmark) oil emulsions, e.g. an emulsion of mineral oil such as BayolF/Arlacel A and water, or an emulsion of vegetable oil, water and an emulsifier such as lecithin; alum, cholesterol, rmlT, cytokines and combinations thereof. The immunogenic component may also be incorporated into liposomes, or conjugated to polysaccharides and/or other polymers for use in a vaccine formulation. Additional substances that can be included in a product for use in the present methods include, but are not limited to one or more preservatives such as disodium or tetrasodium salt of ethylenediaminetetracetic acid (EDTA), merthiolate, and the like.

The subject to which the vaccine is administered is preferably a companion animal, most preferably, a dog or cat.

It is preferred that the vaccine of the invention, when in a vaccine formulation, be present in unit dosage form. For purposes of this invention, an immunogenic amount, when administered comprises about  $1 \times 10^4$  -  $1 \times 10^{13}$  inactivated bacterial cells,  $0.1 \mu\text{g}$  -  $1 \text{ mg}$  of purified protein, or  $0.1 \mu\text{g}$  -  $10 \text{ mg}$  of nucleic acid. In a vaccine formulation containing multiple components, the same or lesser immunogenic amounts can usefully be employed.

Appropriate therapeutically effective doses can be determined readily by those of skill in the art based on the above immunogenic amounts, the condition being treated and the physiological characteristics of the animal. Accordingly, a vaccine preparation provides a dosage of a sterile preparation of an immunogenic amount of the active ingredient(s), where the active ingredient is at least one bacteria, protein, nucleic acid, or any combination thereof. In the presence of additional active agents, these unit dosages can be readily adjusted by those of skill in the art.

A desirable dosage regimen involves administration of at least one dose of desired vaccine composition, where the antigenic content of each fraction is as stated above. Effective doses (immunizing amounts) of the vaccines of the invention may also be extrapolated from dose-response curves derived from model test systems. The mode of administration of the vaccines of the invention can be any suitable route that delivers the vaccine to the host. These include but are not limited to oral, intradermal, intramuscular, intraperitoneal, subcutaneous, intranasal routes, and via scarification (scratching through the top layers of skin, e.g., using a bifurcated needle). However, the vaccine is preferably administered subcutaneously or by intramuscular injection. Other modes of administration

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can also be employed, where desired, such as intradermally, intravenously, intranasally, or intratonsillarly.

Studies have shown that, for each of the above described vaccine compositions, a primary immunization of young animals (after 8 weeks of age) is desirably initiated, with booster doses administered at 12 weeks and 16 weeks of age. Annual re-vaccination is recommended.

The vaccine of the present invention is administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual subject, the site and method of administration, scheduling of administration, subject age, sex, body weight and other factors known to medical practitioners.

The invention further provides kits for the prevention periodontal disease in companion animals. In one embodiment, the kit provides a container comprising a therapeutically effective amount of a composition which prevents periodontal disease in companion animals. Also provided in the same or different container is a pharmaceutically acceptable carrier that may be used in the composition. The kit can additionally include an adjuvant that can be used to aid in creating the response to the composition of the present invention. Also, the kit can include a dispenser for dispensing the composition, preferably in unit dosage form. The dispenser can, for example, comprise metal or plastic foil, such as a blister pack. The kit can be accompanied by a label or printed instructions describing administration of the composition to prevent periodontal disease in a companion animal. Compositions comprising a vaccine composition of the present invention formulated in a pharmaceutically acceptable carrier can also be prepared, placed in an appropriate container, and labeled for treatment of the indicated periodontal condition.

#### **Determination of Vaccine Efficacy**

The specific mechanism of protection induced by the vaccines and vaccine compositions of the present invention is the induction of the antibody and/or cellular immune response in vaccinated animals, as indicated by the *in vivo* animal tests described below.

The bacteria, polynucleotides, polypeptides, vaccines, and vaccine compositions of the present invention may be useful in treating or preventing companion animal periodontal disease, bovine foot rot, coronary heart disease (dogs), or systemic infections (dogs). In addition, the compositions of the present invention may also be useful in treating or preventing certain illnesses in companion animals corresponding to similar illnesses in humans such as coronary heart (or vascular or artery) disease, parotitis, oral malocclusion, gingivitis, periodontitis, stroke, atherosclerosis, hyperlipidemia, increased incidence of pre-term delivery of low birth weight infants, bacterial vaginosis and intrauterine growth retardation (IUGR).

The present invention is further illustrated by the following non-limiting example and accompanying figures.

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**Example****Companion Animal Crevicular Fluid Sample**

Microbial samples were taken from dogs and cats examined at veterinary clinics for periodontal treatment, or dogs examined at either Pfizer Terre Haute or Pfizer Sandwich facilities for normal check-ups. Dogs with periodontal pockets >3mm and cats with periodontal pockets >2mm were included in this study. Dental indices (gingival index and periodontal index) and the periodontal pocket depths were recorded. Individual coarse absorbent paper points (Henry Schein; Melville, NY) were aseptically inserted into the periodontal pocket. Upon removal, the paper points were immediately inserted into vials containing Pre-Reduced Anaerobically Sterile (PRAS) Anaerobic Dental Transport (ADT) Medium (Anaerobe Systems; Morgan Hills, CA).

Vials were transferred into a Bactron IV anaerobic chamber (Sheldon Manufacturing, Cornelius, OR) and processed under 90% N<sub>2</sub>, 5% H<sub>2</sub>, 5% CO<sub>2</sub>. The paper points were aseptically placed into 50 µl of PRAS Brain Heart Infusion (BHI) medium (Anaerobe Systems) and vortexed for 30 seconds. Dilutions of 1:100 and 1:1000 were prepared in BHI medium. Aliquots of 100µl of the 1:100 and 1:1000 dilutions were spread on PRAS Burcella Blood Agar (BRU) plates (Anaerobe Systems). The plates were incubated at 37°C in the anaerobic chamber for five to seven days. The total number of bacterial colonies and the number of Black Pigmented Anaerobic Bacteria (BPAB) colonies were counted. Individual BPAB colonies were transferred to new BRU plates and re-incubated as above.

**Clinical Isolate Characterization**

Each clinical isolate was subjected to a number of biochemical analyses and 16S rRNA DNA sequence analysis, using primers D0056 and D0057 (Seq. ID No. 1 and Seq. ID No. 2; Table 1), to determine genus and species. Individual isolates were streaked on BRU plates. Kanamycin, Vancomycin, and Colistin disks (Anaerobe Systems) were placed on the agar surface to determine the KVC resistance patterns of each isolate. Purified colonies were also subjected to the indole and catalase tests (Anaerobe Systems). Individual isolates were transferred to Egg Yolk Agar (EYA) plates (Anaerobe Systems) in order to determine lipase and lecithinase production patterns. This data is shown in Table 2.

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Table 2. Canine and feline BPAB isolate characterization

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0029	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Y	Y	R	S	R	Y	N	Y	ND	<i>Porphyromonas gingivalis</i>
B0030	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Y	Y	R	S	R	Y	N	Y	ND	<i>Porphyromonas gingivalis</i>
B0031	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Y	Y	R	S	R	Y	N	Y	ND	<i>Porphyromonas gingivalis</i>
B0032	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Light	N	ND	ND	ND	ND	N	N	ND	<i>Porphyromonas circumdentaria</i>
B0033	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Tan	N	ND	ND	ND	ND	N	N	ND	<i>Porphyromonas salivosa</i>
B0034	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Y	Y	R	R	S	Y	Y	Y/N	ND	<i>Prevotella intermedia</i>
B0035	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Y	ND	ND	ND	ND	ND	ND	ND	ND	<i>Prevotella oralis</i>
B0040	NCTC	D	ND	ND	ND	ND	ND	ND	ND	Y	Y	S	S	R	ND	N	Y	Ne	<i>Porphyromonas gingivalis</i>
B0041	Pfizer	D	ND	ND	ND	ND	ND	ND	ND	Y	Y	R	R	R	ND	N	Y	P	<i>Porphyromonas gulae</i>
B0042	Pfizer	D	ND	ND	ND	ND	ND	ND	ND	Y	ND	R	R	R	ND	N	ND	P	<i>Porphyromonas gulae</i>
B0043	Pfizer	D	ND	ND	ND	ND	ND	ND	ND	Y	ND	S	S	R	ND	N	ND	Ne	<i>Porphyromonas gulae</i>
B0044	Pfizer	C	ND	ND	ND	ND	ND	ND	ND	Y	ND	S	S	R	ND	N	ND	P	<i>Porphyromonas gulae</i>
B0045	Pfizer	C	ND	ND	ND	ND	ND	ND	ND	Y	ND	S	S	R	ND	N	ND	P	<i>Porphyromonas gulae</i>
B0046	VHUP1B	D	YRKT	4.5	F	URP4	4	2	2	Y	N	S	S	R	ND	ND	ND	Ne	<i>Porphyromonas cansulci</i>



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Genus/species by 16S rRNA sequence																	
Catalase	Ne	ND	ND	R	R	ND	Y	2	4	URP4	F	4.5	YRKT	D	VHUP1D	B0047	
Lecith.	P	ND	Y	R	R	N	Y	2	4	URP4	F	4.5	YRKT	D	VHUP1E	B0048	
Lipase	P	N	ND	R	R	ND	Y	2	4	URP4	F	4.5	YRKT	D	VHUP1G	B0049	
Indole	Ne	N	ND	R	R	ND	Y	2	4	URP4	F	4.5	YRKT	D	VHUP1H	B0050	
Col	Ne	ND	ND	ND	ND	ND	Y	2	4	URP4	F	4.5	YRKT	D	VHUP1I	B0051	
Vanc	P	ND	ND	R	S	ND	Y	3	5	URP4	M	2.5	DSHA	C	VHUP2A	B0052	
Kan			ND	S	S		Y	3	5	URP4						B0053	
Hemolysis							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2B	B0054	
Pigment							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2C	B0055	
Gingivitis index							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2D	B0056	
Periodontal index							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2E	B0057	
Pocket depth							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2F		
Tooth sampled							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2F		
sex							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2F		
Age							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2F		
Breed							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2F		
Dog/Cat							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2F		
Source							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2F		
Bact. Log #							Y	3	5	URP4	M	2.5	DSHA	C	VHUP2F		

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Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0069	VHUP3A	C	DSHA	12.5	M	ULC	2	1	2	Y	ND	R	R	R	ND	N	Y	P	<i>Porphyromonas gulae</i>
B0070	VHUP3B	C	DSHA	12.5	M	ULC	2	1	2	Y	ND	R	R	R	ND	N	Y	P	<i>Porphyromonas gulae</i>
B0071	VHUP3C	C	DSHA	12.5	M	ULC	2	1	2	Y	ND	R	R	R	ND	N	Y	P	<i>Porphyromonas gulae</i>
B0072	VHUP3D	C	DSHA	12.5	M	ULC	2	1	2	Y	ND	R	R	R	ND	N	N	P	<i>Porphyromonas gulae</i>
B0073	VHUP3E	C	DSHA	12.5	M	ULC	2	1	2	Y	ND	R	R	R	ND	N	N	P	<i>Porphyromonas gulae</i>
B0078	VHUP4A	D	ND	5	F	ULP4	5	3	2	yellow	Y	S	R	R	N	N	Y	Ne	<i>Bacteroides acidofaciens</i>
B0080	VHUP4C	D	ND	5	F	ULP4	5	3	2	yellow	Y	S	S	R	N	N	Y	Ne	<i>Bacteroides acidofaciens</i>
B0083	VHUP4F	D	ND	5	F	ULP4	5	3	2	yellow	N	R	S	R	N	N	Y	Ne	<i>Bacteroides acidofaciens</i>
B0084	DAH1A	D	TPOO	15	F	URCAN	6	3	3	blk	N	R	S	R	Y	Y	N	P	<i>Porphyromonas circumdentaria</i>
B0086	DAH1C	D	TPOO	15	F	URCAN	6	3	3	brown	N	R	R	S	P	Y	N	Ne	<i>Bacteroides fragilis</i>
B0087	DAH1D	D	TPOO	15	F	URCAN	6	3	3	opaque	N	R	R	R	N	N	N	Ne	<i>Porphyromonas circumdentaria</i>
B0089	DAH1F	D	TPOO	15	F	URCAN	6	3	3	dk brn	Y	S	S	R	N	Y	N	P	<i>Porphyromonas gulae</i>
B0090	DAH2A	D	SSHZ	9	M	LRCAN	3	ND	2	lt brn	N	R	S	R	N	Y	N	P	<i>Porphyromonas endodontalis</i>
B0092	DAH2C	D	SSHZ	9	M	LRCAN	3	ND	2	dk brn	Y	S	S	R	N	N	N	P	<i>Porphyromonas gulae</i>
B0093	DAH2D	D	SSHZ	9	M	LRCAN	3	ND	2	dk brn	Y	R	S	R	N	N	N	P	<i>Pasteurella canis</i>

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Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0095	DAH2F	D	SSHZ	9	M	LRCAN	3	ND	2	blk	Y	R	S	R	N	N	N	P	<i>Porphyromonas gulae</i>
B0096	TH1aA	D	ND	ND	M	RPM4	3	ND	ND	lt blk	Y	R	S	R	N	N	Y	P	<i>Porphyromonas gulae</i>
B0097	TH1aB	D	ND	ND	M	RPM4	3	ND	ND	blk	N	R	R	R	N	N	N	P	<i>Porphyromonas circumdentaria</i>
B0098	TH1aC	D	ND	ND	M	RPM4	3	ND	ND	brn	N	S	S	R	N	N	P	P	<i>Porphyromonas cangingivalis</i>
B0103	TH1bB	D	ND	ND	M	LM1	4	ND	ND	blk/wt fans	Y	S	S	R	N	N	Y	Ne	<i>Streptococcus bovis</i> JB1
B0104	TH1bC	D	ND	ND	M	LM1	4	ND	ND	brn	Y	R	R	R	N	Y	P	P	<i>Porphyromonas salivosa</i>
B0105	TH1bD	D	ND	ND	M	LM1	4	ND	ND	brn	Y	R	R	R	N	Y	P	P	<i>Porphyromonas salivosa</i>
B0106	TH1bE	D	ND	ND	M	LM1	4	ND	ND	blk	Y	S	R	S	N	N	Ne	Ne	<i>Porphyromonas denticanis</i>
B0107	TH1bF	D	ND	ND	M	LM1	4	ND	ND	blk	Y	R	R	S	N	N	Ne	Ne	<i>Porphyromonas denticanis</i>
B0109	TH2aB	D	ND	ND	M	LPM4	4	ND	ND	dk brn	N	S	S	R	P	Y	P	P	<i>Porphyromonas cansulci</i>
B0110	TH2aC	D	ND	ND	M	LPM4	4	ND	ND	brn	N	R	R	R	N	Y	P	P	<i>Porphyromonas salivosa</i>
B0111	TH2aD	D	ND	ND	M	LPM4	4	ND	ND	brn	N	S	R	S	P	Y	P	P	<i>Porphyromonas salivosa</i>
B0112	TH2aE	D	ND	ND	M	LPM4	4	ND	ND	brn	Y	R	S	S	P	Y	P	P	<i>Porphyromonas salivosa</i>
B0113	TH2aF	D	ND	ND	M	LPM4	4	ND	ND	blk	Y	R	R	S	P	N	Ne	Ne	<i>Porphyromonas denticanis</i>
	TH2aG	D	ND	ND	M	LPM4	4	ND	ND	yellow					N	N	Ne	Ne	<i>Porphyromonas endodontalis</i>

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Genus/species by 16S rRNA sequence	Catalase	Lecith.	Lipase	Indole	Col	Vanc	Kan	Hemolysis	Pigment	Gingivitis index	Periodontal index	Pocket depth	Tooth sampled	sex	Age	Breed	Dog/Cat	Source	Bact. Log #
<i>Porphyromonas endodontalis</i>	Ne	Y	N	N	R	R	R	Y	dk brn	ND	ND	4	LM1	M	ND	ND	D	TH2bA	B0114
<i>Porphyromonas salivosa</i>	P	N	Y	N	R	R	R	Y	opaque	ND	ND	4	LM1	M	ND	ND	D	TH2bD	B0117
<i>Eubacterium brachy</i>	Ne	Y	N	N	R	S	R	N	yellow	ND	ND	4	LM1	M	ND	ND	D	TH2bE	B0118
<i>Porphyromonas cansulci</i>	Ne	Y	N	N	R	R	R	N	blk	ND	ND	4	LM1	M	ND	ND	D	TH2bF	B0119
<i>Porphyromonas cansulci</i>	Ne	N	N	N	R	S	S	N	blk	ND	ND	4	RM1	M	ND	ND	D	TH2cB	B0121
<i>Porphyromonas endodontalis</i>	P	Y	Y	N	R	R	R	Y	lt brn	ND	ND	4	RM1	M	ND	ND	D	TH2cC	B0122
<i>Porphyromonas endodontalis</i>	Ne	Y	N	N	R	S	R	N	blk	ND	ND	4	RM1	M	ND	ND	D	TH2cD	B0123
<i>Porphyromonas salivosa</i>	P	Y	Y	N	R	R	R	Y	dk brn	ND	ND	4	RM1	M	ND	ND	D	TH2cE	B0124
<i>Porphyromonas endodontalis</i>	Ne	Y	N	N	R	S	R	N	blk	ND	ND	4	RM1	M	ND	ND	D	TH2cF	B0125
<i>Porphyromonas denticanis</i>	Ne	N	N	N	S	R	R	Y	blk	ND	ND	4	RM1	M	ND	ND	D	TH3aA	B0126
<i>Porphyromonas salivosa</i>	P	Y	N	N	R	R	R	Y	brn	ND	ND	4	RM1	M	ND	ND	D	TH3aC	B0128
<i>Porphyromonas denticanis</i>	Ne	N	N	N	S	R	R	Y	blk	ND	ND	4	RM1	M	ND	ND	D	TH3aD	B0129
<i>Porphyromonas salivosa</i>	P	Y	N	N	R	R	R	Y	brn	ND	ND	4	RM1	M	ND	ND	D	TH3aF	B0131
<i>Porphyromonas cansulci</i>	Ne	N	N	N	R	S	R	Y	blk	ND	ND	4	RPM3	M	ND	ND	D	TH3bA	B0132
<i>Porphyromonas salivosa</i>	P	N	Y	N	S	R	R	N	brn	ND	ND	4	RPM3	M	ND	ND	D	TH3bB	B0133
<i>Porphyromonas salivosa</i>	P	N	Y	N	S	R	R	N	brn	ND	ND	4	RPM3	M	ND	ND	D	TH3bC	B0134
<i>Porphyromonas denticanis</i>	Ne	N	N	N	S	R	R	Y	blk	ND	ND	4	RPM3	M	ND	ND	D	TH3bD	B0135

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0136	TH3bE	D	ND	ND	M	RPM3	4	ND	ND	brn	N	R	R	S	P	Y	N	P	<i>Porphyromonas salivosa</i>
B0140	TH3cC	D	ND	ND	M	LM1	4	ND	ND	blk	N	R	R	R	N	Y	Y	Ne	<i>Porphyromonas denticanis</i>
B0142	TH3cE	D	ND	ND	M	LM1	4	ND	ND	opaque	Y	R	R	R	N	Y	Y	P	<i>Porphyromonas salivosa</i>
B0143	TH3cF	D	ND	ND	M	LM1	4	ND	ND	wht	Y	ND	ND	ND	P	N	N	Ne	<i>Eubacterium brachy</i>
B0145	TH4aB	D	ND	ND	M	RM1	4	ND	ND	blk	Y	S	S	R	P	Y	N	P	<i>Porphyromonas gulae</i>
B0146	TH4aC	D	ND	ND	M	RM1	4	ND	ND	lt brn	N	S	S	S	N	Y	N	Ne	<i>Enterococcus gallinarum</i>
B0148	TH4aE	D	ND	ND	M	RM1	4	ND	ND	lt brn	N	R	R	S		Y	N	Ne	<i>Porphyromonas cansulci</i>
B0150	TH4bA	D	ND	ND	M	LM1	4	ND	ND	blk	Y	R	R	S	P	N	N	Ne	<i>Porphyromonas denticanis</i>
B0151	TH4bB	D	ND	ND	M	LM1	4	ND	ND	blk	Y	R	R	S	N	N	N	Ne	<i>Porphyromonas denticanis</i>
B0152	TH4bC	D	ND	ND	M	LM1	4	ND	ND	blk	Y	R	R	S	N	N	N	Ne	<i>Porphyromonas denticanis</i>
B0153	TH4bD	D	ND	ND	M	LM1	4	ND	ND	brn	N	R	R	S	N	Y	N	P	<i>Bacteroides forsythus</i>
B0154	TH4bE	D	ND	ND	M	LM1	4	ND	ND	brn	Y	R	R	S	P	Y	N	P	<i>Porphyromonas salivosa</i>
B0155	TH4bF	D	ND	ND	M	LM1	4	ND	ND	blk	Y	R	R	S	N	N	N	Ne	<i>Porphyromonas denticanis</i>
B0163	TH5bB	D	ND	ND	M	LPM4	4	ND	ND	blk	Y	R	R	S	N	Y	N	Ne	<i>Porphyromonas denticanis</i>
B0164	TH5bC	D	ND	ND	M	LPM4	4	ND	ND	brn	N	S	S	S	P	Y	Y	P	<i>Eubacterium brachy</i>
B0171	TH6aD	D	ND	ND	M	RPM4	7	ND	ND	blk	Y	R	R	S	N	N	N	P	<i>Porphyromonas denticanis</i>
B0172	TH6aE	D	ND	ND	M	RPM4	7	ND	ND	blk	Y	R	R	S	N	N	Y	Ne	<i>Porphyromonas denticanis</i>

Genus/species by 16S rRNA sequence																
	<i>Porphyromonas denticanis</i>	Ne	N	N	S	R	R	Y	blk	ND	6.5	LM1	M	ND	D	TH6bA
	<i>Porphyromonas denticanis</i>	Ne	N	N	S	R	R	Y	opaque	ND	2.5	RPM4	M	ND	D	TH7aD
	<i>Porphyromonas endodontalis</i>	Ne	Y	N	R	R	R	N	blk/brn	ND	4	LM1	M	ND	D	TH7bA
	<i>Porphyromonas canoris</i>	Y	N	N	R	R	R	Y	brn	ND	4	LM1	M	ND	D	TH7bB
	<i>Fusobacterium alocis</i>	Ne	N	N	R	S	R	N	opaque	ND	4	LM1	M	ND	D	TH7bC
	<i>Porphyromonas salivosa</i>	P	Y	Y	R	R	R	Y	brn	ND	4	LM1	M	ND	D	TH7bE
	<i>Porphyromonas salivosa</i>	P	Y	Y	R	R	R	Y	wt	ND	4	LM1	M	ND	D	TH7bF
	<i>Porphyromonas circumdentaria</i>	Ne	Y	Y	R	S	R	Y	blk	ND	3	RM1	M	ND	D	TH8aD
	<i>Porphyromonas salivosa</i>	P	Y	Y	S	R	R	N	lt brn	ND	4	LPM3	M	ND	D	TH9aA
	<i>Porphyromonas gulae</i>	P	N	Y	R	S	R	Y	brn	ND	4	LPM3	M	ND	D	TH9aB
	<i>Campylobacter sputorum</i>	Ne	N	N	S	R	R	Y	blk	ND	4	LPM3	M	ND	D	TH9aD
	<i>Porphyromonas gulae</i>	P	N	Y	R	S	R	Y	dk brn	ND	4	LPM3	M	ND	D	TH9aF
	<i>Porphyromonas cangingivalis</i>	P	N	N	S	S		N	tan	ND	3	RPM3	M	ND	D	TH9bA
	<i>Porphyromonas cansulci</i>	Ne	N	Y	R	R	R	N	blk	ND	3	RPM3	M	ND	D	TH9bB
	<i>Porphyromonas gulae</i>	P	Y	N	R	R	R	Y	brn	ND	3	RPM3	M	ND	D	TH9bC
	<i>Porphyromonas cansulci</i>	Ne	N	Y	R	R	R	N	blk	ND	3	RPM3	M	ND	D	TH9bD

Genus/species by 16S rRNA sequence	<i>Porphyromonas cangingivalis</i>		<i>Campylobacter sputorum</i>		<i>Porphyromonas circumdentaria</i>		<i>Porphyromonas gulae</i>		<i>Porphyromonas circumdentaria</i>		<i>Peptostreptococcus sp. D1</i>	<i>Bacteroides levii</i>	<i>Bacteroides levii</i>	<i>Porphyromonas canoris</i>	<i>Klebsiella oxytoca</i>	<i>Porphyromonas salivosa</i>	<i>Bacteroides forsythus</i>	<i>Porphyromonas salivosa</i>	<i>Porphyromonas circumdentaria</i>
Catalase	P		Ne		P		P		P		Ne	Ne	Ne	P	P	P	Ne	P	Ne
Lecith.	N		N		N		Y		Y		Y	N	N	N	N	Y	Y	Y	Y
Lipase	N		N		N		N		N		Y	Y	Y	N	Y	Y	N	Y	Y
Indole	N		N		N		N		N		N	N	N	P	P	P	N	N	N
Col	R		S		R		R		R		S	R	R	S	R	R	R	R	S
Vanc	S		R		R		R		R		S	R	R	S	S	S	R	R	S
Kan	R		R		R		R		R		R	R	R	R	R	R	R	R	R
Hemolysis	N		N		N		Y		N		N	Y	N	N	Y	Y	N	N	Y
Pigment	tan		opaque		blk		lt brn		blk/brn		yellow	blk	blk	lt brn	brn	mixed	yellow	lt brn	blk
Gingivitis index	ND		ND		ND		ND		ND		ND	ND	ND	ND	ND	ND	ND	ND	ND
Periodontal index	ND		ND		ND		ND		ND		ND	ND	ND	ND	ND	ND	ND	ND	ND
Pocket depth	3		4		4		4		4		4	4	4	2	2	4	4	4	4
Tooth sampled	RPM3		RM1		RM1		RM1		RM1		LM1	RM1	RM1	LPM3	LPM3	RPM4	RPM4	RPM4	RPM4
sex	M		M		M		M		M		M	F	F	F	F	F	F	F	F
Age	ND		ND		ND		ND		ND		ND	ND	ND	ND	ND	ND	ND	ND	ND
Breed	ND		ND		ND		ND		ND		ND	ND	ND	ND	ND	ND	ND	ND	ND
Dog/Cat	D		D		D		D		D		D	D	D	D	D	D	D	D	D
Source	TH9bE		TH10aA		TH10aB		TH10aC		TH10aD		TH10bC	TH11aA	TH11aD	TH11bE	TH11bF	TH12aA	TH12aB	TH12aC	TH12aE
Bact. Log #	B0208		B0210		B0211		B0212		B0213		B0218	B0222	B0225	B0232	B0233	B0234	B0235	B0236	B0238

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0241	TH12bB	D	ND	ND	F	ULPM4	4	ND	ND	wht	N	S	S	R	P	N	N	Ne	<i>Bacteroides acidofaciens</i>
B0242	TH12bC	D	ND	ND	F	ULPM4	4	ND	ND	brn	N	R	R	S	P	Y	N	Ne	<i>Bacteroides acidofaciens</i>
B0243	TH12bD	D	ND	ND	F	ULPM4	4	ND	ND	yellow	N	S	S	R	P	N	N	Ne	<i>Peptostreptococcus sp. D1</i>
B0248	TH13aC	D	ND	ND	M	RPM4	2	ND	ND	blk	Y	R	S	R	N	N	N	Ne	<i>Porphyromonas endodontalis</i>
B0251	TH13aF	D	ND	ND	M	RPM4	2	ND	ND	lt brn	N	S	R	S	N	Y	Y	P	<i>Porphyromonas salivosa</i>
B0258	TH14aA	D	ND	ND	M	URPM2	5	ND	ND	lt brn	Y	R	R	S	N	Y	Y	P	<i>Porphyromonas endodontalis</i>
B0259	TH14aB	D	ND	ND	M	URPM2	5	ND	ND	blk	N	R	S	S	N	N	N	P	<i>Porphyromonas endodontalis</i>
B0260	TH14aC	D	ND	ND	M	URPM2	5	ND	ND	dk brn	N	R	S	S	P	Y	Y	P	<i>Porphyromonas salivosa</i>
B0264	TH14bA	D	ND	ND	M	ULCAN	2	ND	ND	blk	Y	S	S	R	N	N	N	Ne	<i>Porphyromonas denticanis</i>
B0265	TH14bB	D	ND	ND	M	ULCAN	2	ND	ND	blk	Y	R	ND	ND	N	N	N	Ne	<i>Porphyromonas denticanis</i>
B0266	TH14bC	D	ND	ND	M	ULCAN	2	ND	ND	yellow	Y	S	S	R	P	N	N	Ne	<i>Peptostreptococcus sp. D1</i>
B0267	TH14bD	D	ND	ND	M	ULCAN	2	ND	ND	blk	N	R	ND	ND	N	N	N	Ne	<i>Porphyromonas denticanis</i>
B0269	TH14bF	D	ND	ND	M	ULCAN	2	ND	ND	blk	N	R		R	N	ND	ND	P	<i>Porphyromonas denticanis</i>
B0270	TH15aA	D	ND	ND	M	RM1	4	ND	ND	brn	Y	R	R	R	N	N	Y	P	<i>Porphyromonas gulae</i>
B0271	TH15aB	D	ND	ND	M	RM1	4	ND	ND	brn	Y	R	R	R	N	N	Y	P	<i>Porphyromonas gulae</i>
B0272	TH15aC	D	ND	ND	M	RM1	4	ND	ND	gry brn	Y	R	S	R	N	N	Y	P	<i>Porphyromonas gulae</i>
B0273	TH15aD	D	ND	ND	M	RM1	4	ND	ND	blk	Y	R	S	R	N	N	Y	P	<i>Porphyromonas gulae</i>



Genus/species by 16S rRNA sequence	Catalase	Lecith.	Lipase	Indole	Col	Vanc	Kan	Hemolysis	Pigment	Gingivitis index	Periodontal index	Pocket depth	Tooth sampled	sex	Age	Breed	Dog/Cat	Source	Bact. Log #
<i>Porphyromonas gulae</i>	P	Y	N	N	R	S	R	Y	dkbrn	ND	ND	4	RM1	M	ND	ND	D	TH15aE	B0274
<i>Porphyromonas endodontalis</i>	P	N	Y	N	R	S	R	Y	blk	ND	ND	2	LM1	M	ND	ND	D	TH15bD	B0279
<i>Porphyromonas cansulci</i>	P	Y	N	N	R	S	R	ND	brn	ND	ND	4	RM1	F	ND	ND	D	TH16aB	B0283
Unidentified eubacterium	P	N	N	N	S	S	S	ND	blk	ND	ND	4	RM1	F	ND	ND	D	TH16aC	B0284
Unidentified eubacterium	Ne	N	N	N	S	S	S	ND	blk	ND	ND	4	RM1	F	ND	ND	D	TH16aD	B0285
<i>Porphyromonas gulae</i>	P	Y	N	N	S	S	S	ND	brn	ND	ND	4	RM1	F	ND	ND	D	TH16aE	B0286
<i>Porphyromonas circumdentaria</i>	P	N	N	N	R	S	R	ND	brn	ND	ND	4	RM1	F	ND	ND	D	TH16aF	B0287
<i>Porphyromonas circumdentaria</i>	Ne	N	N	P	R	S	R	ND	blk	ND	ND	2	LPM4	F	ND	ND	D	TH16bC	B0290
<i>Porphyromonas gulae</i>	P	Y	N	N	R	S	R	ND	dk brn	ND	ND	2	LPM4	F	ND	ND	D	TH16bD	B0291
<i>Porphyromonas circumdentaria</i>	P	N	N	N	R	S	R	Y	blk	2	2	4	URCAN	F	10	DSHA	C	VHUP5F	B0323
<i>Porphyromonas gulae</i>	P	Y	N	N	R	S	R	Y	brn	3	3	6	URCAN	F	10	COLI	D	DAH6A	B0336
Unidentified eubacterium	P	N	N	N	S	S	S	N	blk	3	3	6	URCAN	F	10	COLI	D	DAH6B	B0337
Unidentified rumen bacterium	P	N	N	N	S	S	S	Y	blk	3	3	6	URCAN	F	10	COLI	D	DAH6F	B0341
<i>Bacteroides acidofaciens</i>	Ne	N	N	P	S	R	S	N	yellow	2	3	5	LM1	M	7.5	SCOT	D	VHUP6A	B0342

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0343	VHUP6B	D	SCOT	7.5	M	LM1	5	3	2	lt brn	N	R	S	R	P	Y	N	Ne	<i>Bacteroides forsythus</i>
B0344	VHUP6C	D	SCOT	7.5	M	LM1	5	3	2	blk	N	ND	ND	ND	N	Y	N	P	<i>Porphyromonas circumdentaria</i>
B0346	VHUP6E	D	SCOT	7.5	M	LM1	5	3	2	brn	N	R	S	S	N	Y	Y	P	<i>Bacteroides forsythus</i>
B0348	VHUP7A	D	CKSP	11	M	ULP2	6	1	2	yellow	N	S	S	R	P	N	N	Ne	<i>Peptostreptococcus</i>
B0353	DAH8B	D	YRKT	11	M	ULCAN	9	2	2	blk	Y	ND	ND	ND	N	N	N	P	<i>Porphyromonas gulae</i>
B0358	DAH19A	D	YRKT	9	M	URPM4	6	3	3	brn	N	S	S	S	P	Y	Y	Ne	<i>Porphyromonas salivosa</i>
B0363	DAH19F	D	YRKT	9	M	URPM4	6	3	3	blk	Y	R	R	S	P	N	N	Ne	<i>Porphyromonas denticanis</i>
B0365	DAH20B	D	DACH	10	F	ULM1	3	3	3	blk	Y	R	R	S	P	N	N	Ne	<i>Porphyromonas denticanis</i>
B0366	DAH20C	D	DACH	10	F	ULM1	3	3	3	blk	Y	R	R	S	P	N	N	Ne	<i>Porphyromonas denticanis</i>
B0367	DAH20D	D	DACH	10	F	ULM1	3	3	3	blk	Y	R	R	S	N	N	N	P	<i>Porphyromonas gulae</i>
B0368	DAH24D	D	MIXB	11	M	LRM1	3	3	2	blk	Y	R	R	S	P	N	N	Ne	<i>Porphyromonas denticanis</i>
B0253	DAH37E	C	DSHA	11	M	URCAN	6	2	3	yel	N	R	S	S	N	N	N	Ne	<i>Bacteroides forsythus</i>
B0255	CSU1B	C	DSHA	17	M	ND	N	ND	ND	lt brn	N	R	S	R	N	Y	Y	P	<i>Tessaracoccus bendigoniensis</i>
B0256	DAH39C	D	ND	ND	M	LRM1	6	2	2	Blk	N	R	R	R	N	N	N	Ne	<i>Bacteroides levii</i>
B0375	UCD2A	D	DACH	11	M	URPM3	5	1	3	brn	N	ND	ND	ND	P	N	N	Ne	<i>Porphyromonas salivosa</i>
B0381	UF1A	C	DSHA	2	F	ULPM3	1	1	2	wt	N	R	R	R	P	N	N	Ne	<i>Porphyromonas denticanis</i>

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0385	UF1E	C	DSHA	2	F	ULPM3	1	1	2	lt brn	N	R	R	S	P	N	N	Ne	<i>Campylobacter sputorum</i>
B0389	UF2C	C	DSHA	2	F	ULPM3	0.5	1	1	brn	N	S	?	R	P	N	N	P	<i>Porphyromonas circumdentaria</i>
B0390	UF2D	C	DSHA	2	F	ULPM3	0.5	1	1	dk brn	Y	ND	ND	ND	P	N	N	P	<i>Staphylococcus warneri partia</i>
B0391	UF2E	C	DSHA	2	F	ULPM3	0.5	1	1	dk brn	Y	ND	ND	ND	P	N	N	P	<i>Salmonella bongori</i>
B0392	UF2F	C	DSHA	2	F	ULPM3	0.5	1	1	brn	N	ND	ND	R	P	N	N	Ne	<i>Clostridium sp.</i>
B0394	UF3B	C	DSHA	2	F	ULPM3	1	1	1	lt brn	N	R	S	S	N	Y	Y	P	<i>Porphyromonas salivosa</i>
B0398	UF3F	C	DSHA	2	F	ULPM3	1	1	1	dk brn	Y	R	S	R	N	N	N	P	<i>Porphyromonas gulae</i>
B0401	UF4C	C	DSHA	2	F	URPM3	1	1	1	yel	N	R	R	R	P	N	N	Ne	<i>Porphyromonas denticanis</i>
B0402	UF4D	C	DSHA	2	F	URPM3	1	1	1	dk brn	Y	ND	ND	ND	P	N	N	P	<i>Porphyromonas gulae</i>
B0403	UF4E	C	DSHA	2	F	URPM3	1	1	1	dk brn	N	S	S	R	N	N	N	P	<i>Porphyromonas gulae</i>
B0411	UF7A	C	DSHA	5	F	ULPM3	1	1	2	dk brn	N	S	S	R	P	N	N	Ne	<i>Globicatella sp.</i>
B0412	UF7B	C	DSHA	5	F	ULPM3	1	1	2	grybrn	N	S	S	S	P	Y	Y	P	<i>Porphyromonas salivosa</i>
B0414	UF7D	C	DSHA	5	F	ULPM3	1	1	2	grybrn	N	S	S	S	P	Y	Y	P	<i>Porphyromonas salivosa</i>
B0416	UF7F	C	DSHA	5	F	ULPM3	1	1	2	brnfan	N	ND	S	R	P	N	N	Ne	Marine snow assoc. bacterium
B0417	UF9A	C	DSHA	ND	F	ULPM3	2	2	2	yel	N	R	R	R	P	N	N	Ne	<i>Porphyromonas denticanis</i>
B0418	UF9B	C	DSHA	ND	F	ULPM3	2	2	2	grybrn	N	R	R	R	P	N	N	Ne	<i>Porphyromonas denticanis</i>

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0421	UF9E	C	DSHA	ND	F	ULPM3	2	2	2	grybm	N	R	R	R	P	N	N	Ne	<i>Porphyromonas denticanis</i>
B0422	UF9F	C	DSHA	ND	F	ULPM3	2	2	2	blk	N	R	R	R	P	N	N	Ne	<i>Porphyromonas denticanis</i>
B0423	UF10A	C	DSHA	ND	F	ULPM3	2	2	2	blk	Y	S	S	R	N	Y	N	P	<i>Porphyromonas gulae</i>
B0427	UF10E	C	DSHA	ND	F	ULPM3	2	2	2	blk	N	ND	ND	ND	N	N	N	P	<i>Porphyromonas gulae</i>
B0428	UF10F	C	DSHA	ND	F	ULPM3	2	2	2	blk	N	R	R	R	P	Y	N	P	<i>Porphyromonas gulae</i>
B0437	UCD4C	D	MSHZ	4	F	LLM1	ND	2	2	brnfan	Y	S	S	S	P	N	N	Ne	<i>Veillonella</i> sp. oral clone X042
B0438	UCD4D	D	MSHZ	4	F	LLM1	ND	2	2	yel	N	ND	ND	ND	N	N	N	Ne	<i>Prevotella oulbra</i>
B0439	UCD4E	D	MSHZ	4	F	LLM1	ND	2	2	lt brn	ND	ND	ND	ND	ND	ND	ND	ND	<i>Lactobacillus rimae</i>
B0440	UCD4F	D	MSHZ	4	F	LLM1	ND	2	2	lt brn	Y	S	S	S	N	N	N	Ne	<i>Streptococcus suis</i>
B0442	UCD5B	D	BOXE	12	F	URI1	3	2	2	ND	ND	ND	ND	ND	P	ND	ND	Ne	<i>Capnocytophaga</i> sp.
B0446	UCD6A	C	DSHA	2	M	LRM1	ND	2	2	ltbrn	N	R	S	R	N	N	N	P	<i>Porphyromonas circumdentaria</i>
B0447	UCD6B	C	DSHA	2	M	LRM1	ND	2	2	blk	N	R	S	S	P	N	N	P	<i>Porphyromonas circumdentaria</i>
B0448	UCD6C	C	DSHA	2	M	LRM1	ND	2	2	blk	N	R	S	R	P	N	N	P	<i>Porphyromonas circumdentaria</i>
B0449	UCD6D	C	DSHA	2	M	LRM1	ND	2	2	bm	N	R	S	?	P	N	N	P	<i>Porphyromonas</i>

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Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0450	UCD6E	C	DSHA	2	M	LRM1	ND	2	2	brn	N	R	S	R	N	N	Ne		<i>circumdentaria</i>
B0452	UCD6G	C	DSHA	2	M	LRM1	ND	2	2	blk	N	R	S	R	P	N	P		<i>Porphyromonas circumdentaria</i>
B0453	UCD6H	C	DSHA	2	M	LRM1	ND	2	2	blk	N	R	S	R	N	N	P		<i>Porphyromonas circumdentaria</i>
B0456	UCD7B	D	POOD	8	F	URCAN	ND	ND	ND	blk	N	R	R	S	N	N	P		<i>Porphyromonas denticanis</i>
B0457	UCD7C	D	POOD	8	F	URCAN	ND	ND	ND	brn	N	R	R	S	P	N	Ne		<i>Porphyromonas denticanis</i>
B0458	UCD7D	D	POOD	8	F	URCAN	ND	ND	ND	yel	N	R	S	R	P	N	Ne		<i>Bacteroides acidofaciens</i>
B0463	UCD8C	C	DLHA	6	M	LLP4	ND	1	ND	brn	N	S	S	R	P	N	P		<i>Peptostreptococcus sp.</i>
B0473	UCD10A	D	WHWT	10	M	URP4	3	2	2	wht	N	R	R	S	P	ND	Ne		<i>Bacteroides acidofaciens</i>
B0474	UCD10B	D	WHWT	10	M	URP4	3	2	2	wyel	N	R	ND	S	P	ND	Ne		<i>Bacteroides acidofaciens</i>
B0476	UCD10D	D	WHWT	10	M	URP4	3	2	2	wht	N	R	R	R	P	ND	Ne		<i>Bacteroides acidofaciens</i>
B0477	UCD10E	D	WHWT	10	M	URP4	3	2	2	brn	Y	R	R	R	P	ND	P		<i>Porphyromonas salivosa</i>
B0478	UCD10F	D	WHWT	10	M	URP4	3	2	2	brn	Y	R	R	R	P	ND	Ne		<i>Porphyromonas salivosa</i>

Abbreviations: D, Dog; C, Cat; NA, Not applicable; ND, Not determined; M, Male; F, Female; Y, Yes; N, No; P, Positive; Ne, Negative

The isolates were typed based on their 16S rRNA DNA sequence. Individual, well-isolated colonies were utilized as template for polymerase chain reactions (PCR) amplification of the 16S rRNA region using primers D0056 and D0057 (Seq. ID No. 1 and Seq. ID No. 2; Table 1) in triplicate. The PCR was carried out in 50 µl reaction volumes containing 1 x PCR buffer (Life Technologies; Rockville, MD), 1.0 mM MgCl<sub>2</sub>, 1.25 µM each primer, 300 µM each deoxy-NTP, and 2.5 U Platinum *Pfx* DNA Polymerase (Life Technologies). The following PCR cycle conditions were utilized: a two minute denaturation step at 94°C; 30 cycles of denaturation at 94°C for 40 seconds, annealing at 60°C for 40 seconds, and extension at 72°C for one minute; a final extension step at 72°C for two minutes; and a final cooling step to 4°C. A GeneAmp 9700 thermocycler (Perkin Elmer Applied Biosystems; Foster City, CA) was utilized for all PCR amplifications.

The resulting PCR products were purified using the PCR preps kits (Promega Corp.; Madison, WI) and pooled by isolate. The purified PCR products were then desalted by drop analysis against 25 ml sterile water using a 0.025 µm nitrocellulose filter (Millipore Corp.; Bedford, MA). The purified, desalted PCR products were subjected to DNA sequence analysis using the DyeDeoxy termination reaction on an ABI automated DNA sequencer (University of Texas Genetics Core Facility, Houston, TX and Lark Technologies Inc., Houston, TX). Synthetic oligonucleotide primers D0056, D0057, PFZ175-AP1, PFZ175-AP2, and PFZ175-AP3 (Seq. ID No. 1-5, respectively; Table 1) were used to obtain double stranded DNA sequence. The resulting DNA sequences were used to search publicly available DNA databases using a BLAST-N program publicly available from The National Center for Biotechnology Information, USA.

The bacterial isolates were typed based on the closest match identified by database searches. The B106 isolates did not have a precise match. The nearest match was with an uncultured bacterial type that was identified by random PCR of human periodontal pocket material. This isolate was referred to as *Porphyromonas denticanis* strain B106. A complete listing of all the isolates and their respective characteristics is located in Table 2. The top nine most frequently isolated strains are exemplified by the following isolates: *P. gulae* B43 (dog sample Sandwich 4), *P. cansulci* B46 (dog sample VHUP 1B), *P. circumdentaria* B52 (cat sample VHUP 2A), *P. gulae* B69 (cat sample VHUP 3A), *P. circumdentaria* B97 (dog sample TH 1bC), *P. cangingivalis* B98 (dog sample TH 1aC), *P. salivosa* B104 (dog sample TH 1bC), *P. denticanis* B106 (dog sample TH 1bE), and *P. endodontalis* B114 (dog sample TH 2bA).

The distribution of isolates is shown in Table 3.

**Table 3.** Summary of the number of dogs and cats identified to harbor indicated bacterial species.

Isolate	# dog isolates	# dogs	% positive dogs	# cat isolates	# cats	% positive
<i>Porphyromonas gulae</i>	27	16	31	8	6	38
<i>Porphyromonas salivosa</i> (macacae)	27	17	33	3	2	13
<i>Porphyromonas denticanis</i>	24	15	29	0	0	0
<i>Porphyromonas cansulci</i>	12	8	15	0	0	0
<i>Porphyromonas endodontalis</i>	11	8	15	0	0	0
<i>Porphyromonas circumdendaria</i>	10	8	15	15	4	25
<i>Bacteroides acidofaciens</i>	10	5	10	0	0	0
<i>Bacteroides forsythus</i>	4	3	6	1	1	6
<i>Porphyromonas cangingivalis</i>	3	2	4	0	0	0
<i>Bacteroides levii</i>	3	2	4	0	0	0
<i>Eubacterium brachy</i> ATCC33089	3	3	6	0	0	0
<i>Peptostreptococcus sp. D1</i>	3	4	8	1	1	6
Unidentified eubacterium	3	2	4	0	0	0
<i>Porphyromonas canoris</i>	2	2	4	0	0	0
<i>Campylobacterium sputorum</i>	2	2	4	1	1	6
<i>Porphyromonas gingivalis</i>	1	1	2	0	0	0
<i>Bacteroides fragilis</i>	1	1	2	0	0	0
Uncultured bacterium SHA-54	1	1	2	0	0	0
Uncultured bacterium SHA-219	1	1	2	0	0	0
<i>Pasteurella canis</i>	1	1	2	0	0	0
<i>Streptococcus bovis JB1</i>	1	1	2	0	0	0
<i>Enterococcus gallinarum</i>	1	1	2	0	0	0
<i>Fusobacterium alocis</i>	1	1	2	0	0	0
<i>Klebsiella oxytoca</i>	1	1	2	0	0	0
Unidentified rumen	1	1	2	0	0	0

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Isolate		# dog isolates	# dogs	% positive dogs	# cat isolates	# cats	% positive
<i>bacterium</i>							
Uncultured	bacterium	0	0	0	6	3	19
AF132259							
	<i>Prevotella oulora</i>	0	1	2	0	0	0
	<i>Tessatacoccus</i>	0	0	0	1	1	6
<i>bendigoniensis</i>							
	<i>Staphylococcus warneri</i>	0	0	0	1	1	6
	<i>Salmonella bongori</i>	0	0	0	1	1	6
	<i>Clostridium sp.</i>	0	0	0	1	1	6
	<i>Globicatella sp.</i>	0	0	0	1	1	6
Marine snow	associated	0	0	0	1	1	6
<i>bacterium</i>							
	<i>Veillonella sp. oral clone</i>	0	1	2	0	0	0
X042							
	<i>Lactobacillus rimae</i>	0	1	2	0	0	0
	<i>Streptococcus suis</i>	0	1	2	0	0	0
	<i>Capnocytophaga sp.</i>	0	1	2	0	0	0

The isolates listed above represent those species that were most frequently identified and present in the highest percentages of dogs or cats.

- 5 The following companion animal periodontal isolates were deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA, 20110, USA, on August 9, 2001: *P. gulae* B43 (PTA-3618), *P. cansulci* B46 (PTA-3619), *P. circumdentaria* B52 (PTA-3620), *P. gulae* B69 (PTA-3621), *P. circumdentaria* B97 (PTA-3622), *P. cangingivalis* B98 (PTA-3623), *P. salivosa* B104 (PTA-3624), *P. denticanis* B106 (PTA-3625), and *P. endodontalis* B114 (PTA-3626).

#### 10 CULTURE CONDITIONS FOR PORPHYROMONAS SP.

- Since the standard growth media for *Porphyromonas sp.* (Brain Heart Infusion (BHI) and Chopped Meat Carbohydrate (CMC) media) contain animal product, which are not amenable for vaccine production, a growth medium that does not contain these ingredients was sought. Various media compositions, with and without the addition of hemin and vitamin K, were tested for their ability to support growth equivalent to that of growth of BHI or CMC.
- 15 Both the PYG-complete medium and ME-complete medium supported the growth of *P. gulae* B43 (PTA-3618) to a level equivalent to that of BHI (Figure 1). The PYG-complete medium was chosen as the *P. gulae* B43 (PTA-3618) growth medium due to its ability to yield high



density cultures during fermentation. This medium contains the following ingredients: 3% phytone (Becton Dickinson; Cockeysville, MD), 0.3% yeast extract (Becton Dickinson), 0.3% glucose (Sigma Corp.; St. Louis, MO), 0.05% sodium thioglycollate (Becton Dickinson), 0.5% sodium chloride (Sigma Corp.), 5 µg/ml hemin (Sigma Corp.) (added after autoclaving), 0.5 µg/ml menadione (Sigma Corp.) (added after autoclaving), and 0.2% sodium bicarbonate (Sigma Corp.), pH 7.0.

*P. gulae* B43 (PTA-3618) was routinely cultivated on Brucella blood agar plates (Anaerobe Systems) or in complete PYG medium or BHI at 37°C in a Bactron IV anaerobic chamber (Shel Labs; Cornelius, OR) under 90% N<sub>2</sub>, 5% CO<sub>2</sub> for three to five days (plates) or 24 to 48 hours (liquid cultures). For whole cell bacterin preparation, *P. gulae* B43 (PTA-3618) was cultivated in a BioFlo 3000 Bioreactor using 5 liters of PYG complete medium. The culture medium in the vessel was rendered anaerobic by sparging with 95 – 99.5% N<sub>2</sub> and 0.5 – 5% CO<sub>2</sub> immediately after autoclaving. The reduced culture medium was seeded with 0.02% of *P. gulae* B43 (PTA-3618) stock and cultivated at 37°C with an agitation rate of 100 rpm and the pH maintained at 7.0 by the automatic addition of NaOH. During cultivation, the vessel was periodically sparged with both N<sub>2</sub> and CO<sub>2</sub>. The bacterial cells were collected after 36 to 48 hours at an OD<sub>600</sub> of 2.0 to 3.5 while cells were still undergoing logarithmic growth.

#### **Pathogenicity Testing of Clinical Isolates**

The nine isolates (*P. gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106, and *P. endodontalis* B114) were tested for their pathogenicity in the mouse periodontal bone loss model. Three-week-old, age-matched male Balb/c CyJ mice (Jackson Laboratories; Bar Harbor, ME) with estimated weights of 14-15 grams were utilized for this study. The animals were housed in positive pressure, barrier cage units. Food pellets, standard for the species, and water were provided *ad libitum* throughout the experiment. The bedding utilized was granular Bed O'Cobbs to minimize impaction in the gingival tissues. Following receipt, all animals were acclimatized for five to seven days. To reduce competing oral flora, animals were placed on a mixture of sulfamathoxazole and trimethoprim (10 ml drinking water; approximately 2 mg and 0.4 mg/ml, respectively) for ten days followed by a five-day washout period. Serum samples were taken from each mouse tail vein bleed. The animals were infected with 0.5 ml suspension of approximately  $1 \times 10^{10}$  cfu/ml of the appropriate bacterial strain in 1% carboxymethylcellulose by gavage. Additional drops were placed in the oral cavity. This infection was repeated two more times for a total of three times (Monday, Wednesday, and Friday).

Day 1 of the experiment was defined as the Tuesday following the first infection. All animals were sacrificed on Day 2. Post-infection serum was collected, as were microbial

samples. The jaws of each mouse were defleshed, stained, and scored for horizontal bone loss microscopically. The scoring was repeated three times to reduce operator error. The average bone loss is expressed as the average bone loss/site/jaw in mm. Statistical analysis of the resulting data was done with Systat (version 9), SigmaStat (version 2), and SigmaPlot (version 2000) available from SPSS Science Inc. (Chicago, IL). Table 4 shows the numerical results for the top nine isolates.

Table 4. Summary of the mouse periodontal disease pathogenicity trial.

Isolate	Number of mice	Source of bacteria	Mean Bone Loss (mm)	Std. Deviation	SEM
Sham	32	N/A	0.0843	0.0118	0.00211
<i>P. gingivalis</i> 53977	16	Human	0.106	0.0139	0.00347
<i>P. gingivalis</i> W50	16	Human	0.0948	0.0116	0.0029
<i>P. gingivalis</i> B40 A	16	Dog	0.106	0.0138	0.00357
<i>P. gingivalis</i> B40 B	16	Dog	0.115	0.0114	0.00284
<i>P. gulae</i> B43	16	Dog	0.112	0.0163	0.00407
<i>P. cansulci</i> B46	16	Dog	0.101	0.014	0.00362
<i>P. circumdentaria</i> B52	16	Cat	0.0924	0.00836	0.00209
<i>P. gulae</i> B69	16	Cat	0.114	0.0129	0.00322
<i>P. circumdentaria</i> B97	16	Dog	0.0855	0.0143	0.00368
<i>P. cangingivalis</i> B98	16	Dog	0.111	0.0136	0.0034
<i>P. salivosa</i> B104	16	Dog	0.102	0.0107	0.00286
<i>P. denticanis</i> B106	16	Dog	0.124	0.0167	0.00417
<i>P. endodontalis</i> B114	16	Dog	0.0994	0.0223	0.00557

Each of these yielded statistically significant bone loss in this model.

Figure 2 graphically shows the net bone loss. The mean alveolar bone levels (cementoenamel junction – alveolar bone crest) were obtained at 14 maxillary sites in mm, and the mean value for each jaw was determined. For each experimental group, the mean values for each jaw were summed and the group mean derived by dividing by the total number of animals in that group.

Figure 3 graphically shows the comparison of net bone loss. The mean alveolar bone levels (cementoenamel junctions – alveolar bone crest) were obtained at 14 maxillary sites in mm, and the mean value for each jaw was determined. For each experimental group, the mean values for each jaw were summed and the group mean derived by dividing by the total number of animals in that group. The net bone loss was determined by subtracting the sham infected mean values from each experimental groups. The data is presented as a percentage

of the positive control group (*P. gingivalis* 53977) which is set at 100%. *P. gingivalis* W50 is a poorly fimbriated strain that has reduced virulence in this animal model.

These data indicate that the following clinical isolates are capable of producing high levels of bone loss in the mouse model of periodontal disease: *P. gulae* B43 (PTA-3618), *P. gulae* B69 (PTA-3621), *P. cangingivalis* B98 (PTA-3623) and *P. denticanis* B106 (PTA-3625). The following clinical isolates yielded moderate bone loss in the mouse periodontal model: *P. cansulci* B46 (PTA-3619), *P. salivosa* B104 (PTA-3624), and *P. endodontalis* B114 (PTA-3626). The following clinical isolates yielded minimal bone loss in the mouse periodontal model: *P. circumdentaria* B52 (PTA-3620) and *P. circumdentaria* B97 (PTA-3622). While varying amounts of bone loss were observed between the clinical isolates, it should be noted that in each case, the amount of bone loss observed was well above what was observed in the sham infected mice. Based on these data, it can be concluded that each of the top nine clinical isolates is capable of causing periodontal disease either alone or in concert with other bacteria.

#### **PREPARATION OF BACTERIAL CELLS AND GENOMIC DNA**

*Porphyromonas spp.* were anaerobically cultivated in BHI or complete PYG at 37°C for 48 hours. Cells from a 1-3 ml culture were pelleted by centrifugation, washed once in an equal volume of anaerobic PBS, re-centrifuged, and re-suspended in 1/10 volume anaerobic PBS.

Genomic DNA was purified from 5 ml cultures of *Porphyromonas spp.* that were anaerobically cultivated in BHI or complete PYG at 37°C for 48 hours. The Wizard Genomic DNA Extraction kit (Promega Corp.) was utilized for all genomic DNA preparations.

#### **CLONING OF THE FIMBRIAL GENE FROM CLINICAL ISOLATES**

The *fimA* gene was PCR amplified from genomic DNA isolated from the top ten clinical isolates using combinations of the following PCR primers D0067 (forward; Seq. ID No. 6), D0078 (forward; Seq. ID No. 8), D0097 (forward; Seq. ID No. 9), D0068 (reverse; Seq. ID No. 7) and D0098 (reverse; Seq. ID No. 10). The PCR was carried out in 50 µl reaction volumes containing 1x PCR buffer (Life Technologies), 1.0 mM MgCl<sub>2</sub>, 1.25 µM each primer, 300 M each deoxy-NTP, and 2.5 U Platinum *Pfx* DNA Polymerase (Life Technologies). The following PCR cycle conditions were utilized: a two minute denaturation step at 94°C; 30 cycles of denaturation at 94°C for 40 seconds, annealing at 60°C for 40 seconds, and extension at 72°C for 1.5 minutes; a final extension step at 72°C for five minutes; and a final cooling step to 4°C. A GeneAmp 9700 thermocycler (Perkin Elmer Applied Biosystems; Foster City, CA) was utilized for all PCR amplifications. The amplified products were visualized on a 1.2% E-gel (Invitrogen; Carlsbad, CA).

The PCR products were A-tailed using 10 units of KlenTaq polymerase (Ab Peptides, Inc.; St. Louis, MO) for five minutes at 72°C. The resultant products were immediately T-tail

cloned into the pCR2.1-TOPO vector (Invitrogen) using the manufacturer's protocol and transformed into *E. coli* Top10F' (Novagen; Madison, WI). Transformants harboring recombinant plasmids with the correct insert DNA were identified by a combination of colony PCR, restriction enzyme digestion, and DNA sequence analysis using DyeDeoxy termination reactions on an ABI automated DNA sequence (Lark Technologies, Inc.). Synthetic oligonucleotide primers (Seq. ID No. 6, 7, 8, 11-42) were used to obtain double stranded DNA sequence.

#### **CLONING OF THE *P. GULAE* B43 *FIM*A GENE INTO EXPRESSION PLASMIDS**

For the purpose of high-level protein expression, the *P. gulae* B43 (PTA-3618) *fimA* gene was cloned into the pBAD/HisA expression vector (Invitrogen). Genomic DNA was purified from a 5 ml culture of *P. gulae* B43 in BHI incubated at 37°C for two days anaerobically using the genomic DNA extraction kit (Promega Corp.). The *fimA* gene was PCR amplified using primers D0097 and D0098 (Seq. ID No. 9 and Seq. ID No. 10) in triplicate. The PCR was carried out in 50 ul reaction volumes containing 1 X PCR buffer (Life Technologies), 50 ng *P. gulae* B43 genomic DNA, 1.0 mM MgCL<sub>2</sub>, 1.25 µM each primer, 300 µM each deoxy-NTP, and 2.5 U Platinum Pfx DNA Polymerase (Life Technologies, USA).

The following PCR cycle conditions were utilized: a two minute denaturation step at 94°C; five cycles of denaturation at 94°C for 40 seconds, annealing at 58°C for 40 seconds, and extension at 72°C for 1.5 minutes; 30 cycles of denaturation at 94°C for 40 seconds, annealing at 65°C for 40 seconds, and extension at 72°C for 1.5 minutes; a final extension step at 72°C for five minutes; and a final cooling step to 4°C. A GeneAmp 9700 thermocycler (Perkin Elmer Applied Biosystems) was utilized for all PCR amplifications. The PCR products were purified using PCR prep kits (Promega Corp.). The purified PCR products and pBAD/HisA were double digested with *Hind*III and *Xho*I for three hours at 37°C. Half way through the digestion, five units of shrimp alkaline phosphatase (SAP) (Amersham Pharmacia Biotech, Inc.: Piscataway, NJ) were added to the vector digestion. The digested DNA's were purified using the DNA Clean-Up kit (Promega Corp.). The purified *Hind*III/*Xho*I digested PCR products were ligated into *Hind*III/*Xho*I digested, SAP treated pBAD/HisA with the T4 DNA Ligase enzyme (Life Technologies) in the presence of 1 X T4 DNA ligase buffer at 16°C for 18 hours. A portion of the resulting ligation mixture was transformed into competent *E. coli* Top10F' cells (Novagen). A recombinant plasmid, pBAD:B43fimA4, was found to contain the *fimA* gene in the correct orientation. The resulting recombinant FimA contains a terminal, vector-encoded sequence

(MGGSHHHHHHGMSMTGGQMGRDLYDDDDKDRWGSELEICSQYHMG I, SEQ ID NO: 135), followed by the mature portion of FimA beginning at asparagine-20. This plasmid was transformed into competent *E. coli* BL21 cells (Novagen) for further protein expression analysis.

### **EXPRESSION AND PURIFICATION OF THE RECOMBINANT FIMA PROTEIN**

A frozen working stock of the *E. coli* BL21/pBAD:B43fimA4 was thawed, seeded at a 1:5000 dilution into Luria broth containing 100 µg/ml ampicillin (1% tryptone, 0.5% yeast extract, 0.5% NaCl), and grown in a 5 liter working volume BioFlo 3000 Bioreactor (New Brunswick Scientific; Edison, NJ) at 37°C with a 100 rpm agitation rate until  $A_{625}$  was 2.5-3.5. L-arabinose was then added to the culture at a final concentration of 0.1% to induce FimA expression. The culture was incubated for an additional three hours. Expression of the recombinant FimA was detected by SDS-PAGE and Western blot analysis using anti-Express serum (Invitrogen) (Figure 4). The recombinant FimA protein had a predicted molecular mass of 45 kDa.

Wet cells of the *E. coli* BL21 transformant expressing recombinant FimA from the 5 liter fermentation were harvested by centrifugation and re-suspended in phosphate-buffered saline. The cells were mechanically lysed. Following centrifugation, the pellet was discarded. The supernatant was passed over a  $Ni^{2+}$ -affinity column, and eluted off using an imidazole gradient. Fractions containing the recombinant protein were pooled, dialyzed to remove the imidazole, and filter-sterilized using a 0.2 µm filter.

### **CLONING OF THE *OPRF* GENE FROM CLINICAL ISOLATES**

Based on sequences of the *P. gingivalis* strain W50 *oprF* homolog, gene PG32 (Genbank accession number AF175714), oligonucleotide primers D0086 (SEQ ID No. 43), D0087 (SEQ ID NO. 44), and KWK-Pg-03 (SEQ ID NO. 45) were designed and synthesized (Life Technologies). For PCR, primer D0086 (SEQ ID NO. 43) was used in conjunction with either D0087 (SEQ ID NO. 44) or KWK-Pg-03 (SEQ ID NO. 45) in 1 X PC2 buffer (Ab Peptides), 200 µM each dNTP, 7.5 U KlenTaq1 (Ab Peptides) and 0.15 U cloned *Pfu* (Stratagene; La Jolla, CA) thermostable polymerases in a 50 µl final sample volume. Reactions were performed in triplicate using either a washed cell suspension or purified genomic DNA as template from *P. gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106, and *P. endodontalis* B114. Amplification was carried out as follows: denaturation (94°C, 9 minutes); 30-40 cycles of denaturation (94°C, 30 seconds), annealing (55-60°C, 30 seconds), and polymerization (72°C, 1.5 minutes); followed by a final extension at 72°C for seven minutes.

For polymerase chain amplification of the *oprF* homolog from *P. cangingivalis* B98, primer KWK-Ps-04b (SEQ ID No. 81) was used in conjunction with KWK-Ps-06b (SEQ ID No. 83). For amplification of the homolog from *P. salivosa* B104, primer KWK-Ps-04b (SEQ ID No. 81) was used with KWK-Ps-05b (SEQ ID No. 82). For amplification of the gene from *P. denticanis* B106, primer KWK-Ps-02 (SEQ ID No. 79) was used with KWK-Ps-03 (SEQ ID No. 80). Reactions were performed in triplicate using purified chromosomal DNA as template

from strains *P. cangingivalis* B98, *P. salivosa* B104, and *P. denticanis* B106. Amplification was carried out as follows: denaturation (94°C, 9 minutes); 30-35 cycles of denaturation (94°C, 30 seconds), annealing (61-72°C, 30 seconds), and polymerization (72°C, 1.5 minutes); this was followed by a final extension at 72°C for 7 minutes.

5 The PCR amplified gene products were visualized by separation on a 1.0% agarose gel (Sigma). The PCR products were purified using a QIAquick™ PCR Purification kit (Qiagen; Valencia, CA), and each set of triplicate samples pooled. These fragments were then sequenced directly in an attempt to avoid the introduction of sequence artifacts due to mutations that arise during PCR amplification and subsequent cloning steps. The pooled  
10 mixtures were then subjected to direct sequence analysis using DyeDeoxy termination reaction on an ABI automated DNA sequencer (Lark Technologies). Synthetic oligonucleotide primers (SEQ ID NO. 46-75) were used to sequence both DNA strands of the amplified products.

The nucleotide sequences encoding the OprF homolog from *P. gulae* B43, *P.*  
15 *cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106, and *P. endontalis* B114 are depicted in SEQ ID NO. 111 to 119. Sequence corresponding the 5' and 3' primers used for PCR amplification of each gene was removed, as it may not represent the actual sequence of the gene in each of the respective strains.  
20 The ORFs encoded by SEQ ID NO.111 to 119 are shown in SEQ ID No. 120 to 128, respectively. For each of the encoded ORFs, the amino terminal sequence, even when that encoded by the 5' primer was excluded, still maintained characteristics of a prokaryotic signal sequence (von Heijne, 1985, J. Mol. Biol. 184:99-105; Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G., 1997 Protein Engineering, 10: 1-6). Each ORF was compared against  
25 existing nucleotide and protein databases using the Basis Local Align Search Tool (BLAST) programs (Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990, J. Mol. Biol. 215:403-410). The entry with which each shared the greatest homology was PG32 from *P. gingivalis*.

#### **CLONING OF THE *P. GULAE* B43 *OPRF* GENE INTO EXPRESSION PLASMIDS**

30 For the purpose of recombinant protein expression, the gene encoding OprF was cloned with the sequence encoding its signal peptide. *OprF* was amplified from *P. gulae* B43 using oligonucleotide primers KWK-Pg-06 (SEQ ID NO. 76) and KWK-Pg-03 (SEQ ID NO. 45). For polymerase chain amplification, duplicate 50µl reactions were set up containing chromosomal DNA as template, 1 X PC2 buffer, 200 µM each dNTP, 50 pMol each primer,  
35 7.5 U KlenTaq1 and 0.15 cloned *Pfu* thermostable polymerase. Amplification was carried out as follows: denaturation (94°C, nine minutes); 30 cycles of denaturation (94°C, 30 sec), annealing (60°C, 30 sec), and polymerization (72°C, 1.5 min), followed by a final extension at

72°C for 7 minutes. Following amplification, the samples were purified (QIAquick™ PCR Purification kit) and pooled. The purified PCR product was cloned directly into the TA cloning site of both pBAD-TOPO and pBAD/Thio-TOPO (Invitrogen). The ligand products were transformed into Max Efficiency *E. coli* DH5α cells. The predicted amino terminal sequence of the encoded protein expressed from pBAD-TOPO:OprF consists of the vector-encoded sequence MGS GSGDDDDKLALM (SEQ ID NO: 136) followed immediately by the sequence beginning at glutamine-13 of OprF (SEQ ID No. 120). A clone containing the appropriate plasmid was identified, and purified plasmid was isolated from a small-scale broth culture using a QIAprep Spin Miniprep kit (Qiagen). This plasmid was transformed into *E. coli* BL21 cells (Novagen), and a clone was identified that contained the appropriate plasmid.

The predicted amino terminal sequence of the encoded fusion protein expressed from pBAD/Thio-TOPO: *oprF* should consist of the thioredoxin protein and a 14 amino acid residue linker followed immediately by the sequence beginning at glutamine-13 of OprF (SEQ ID NO. 120). A clone containing the appropriate plasmid was identified, and purified plasmid was isolated from a small-scale broth culture using a QIAprep Spin Miniprep kit. This plasmid was transformed into *E. coli* BL21 cells, and a clone was identified that contained the appropriate plasmid.

The *oprF* gene lacking the sequence encoding the signal peptide was also cloned into two different λ expression plasmids. Both of these plasmids encode the temperature-sensitive λ repressor *cI857*, which inhibits expression from λ promoters at 30°C. At 42°C, the repressor is inactivated and expression from the λ promoter is enabled, yielding high-level transcription and translation. For cloning into these vectors, *oprF* was amplified from *P. gulae* B43 using oligonucleotide primers KWK-Pgu-14 (SEQ ID NO. 77) and KWK-Pgu-15 (SEQ ID NO. 78). For polymerase chain amplification, duplicate 50 µl reactions were set up containing washed *P. gulae* B43 cells as template, 1 X PC2 buffer, 200 µM each dNTP, 50 pMol each primer, 7.5 U KlenTaq1 and 0.15 U cloned *Pfu* thermostable polymerases. Amplification was carried out as follows: denaturation (94°C, nine minutes); 45 cycles of denaturation (94°C, 30 seconds), annealing (55°C, 30 seconds), and polymerization (72°C, 1.5 minutes), followed by a final extension at 72°C for seven minutes. Following amplification, the samples were pooled and digested with restriction enzymes, generating overhangs compatible with the plasmids which had also been linearized using the same enzymes. Following restriction digestion, the PCR fragment and plasmids were purified (QIAquick™ PCR Purification kit; Qiagen Corp.), ligated, and transformed into *E. coli* DH5α cells (Novagen). The predicted amino terminal consisted of the vector-encoded sequence MGT TTT TTT SLHM (SEQ ID NO: 137) followed immediately by the sequence beginning at Glutamine-13 of OprF (SEQ ID NO. 120). The protein expressed from the second plasmid would consist only a vector-encoded Met followed by Glutamine-13 of OprF (SEQ ID NO: 120). Clones containing the appropriate

plasmids were identified, and plasmids were isolated from small-scale broth cultures using QIAprep Spin Miniprep kits (Qiagen Corp.). These plasmids were transformed into *E. coli* BL21 cells, and separate clones were identified that contained the appropriate plasmids.

#### **EXPRESSION AND PURIFICATION OF THE RECOMBINANT OPRF PROTEIN**

5 *E. coli* BL21 cells that express recombinant OprF (fused at its N-terminus to SEQ ID NO: 137) were utilized for expression studies. A frozen stock was thawed, seeded at a 1:5000 dilution into 2 X YT medium containing 50 µg/ml kanamycin sulfate (1.6% tryptone, 1% yeast extract, 0.5 NaCl), and grown in a 5 liter working volume BioFlo 3000 Bioreactor (New Brunswick Scientific; Edison, NJ) at 29°C with a 100 rpm agitation rate until  $A_{625}$  was  
10 2.5-3.5. The cultures were then shifted to 42°C to induce OprF expression. The culture was incubated for an additional 3 hours. Aliquots were removed at various time points, centrifuged, and re-suspended in reducing sample buffer. All samples were analyzed on a 10% NuPAGE gel (Invitrogen, USA) (Figure 5).

Wet cells of the *E. coli* BL21 transformant expressing recombinant OprF from the 5 liter fermentation were harvested by centrifugation and re-suspended in phosphate-buffered  
15 saline. The cells were mechanically lysed. Following centrifugation, the pellet was discarded. The supernatant was passed over an ion exchange column, and eluted off using a NaCl gradient. Fractions containing the recombinant protein were pooled, dialyzed to remove the NaCl, and filter-sterilized using a 0.2 µm filter.

#### **WHOLE CELL BACTERIN PREPARATION**

20 A 5 liter batch of *P. gulae* B43 was grown in a fermentor as described above and split into 1 liter portions. The cells in each 1 liter fraction ( $4.4 \times 10^{12}$  total *P. gulae* B43 cells) were inactivated by the following treatments: exposure to 0.4% formalin for 24 hours at 23°C, exposure to 10 mM binary ethylene-imine (BEI) at pH 8.5 for 48 hours at 37°C, heating to  
25 60°C for 30 minutes on two consecutive days, and exposure to air for 48 hours. Following the BEI treatment, the BEI was inactivated by treatment with 50 mM sodium thiosulfate. The cells were collected by centrifugation. The resultant cells pellets were re-suspended in 220 ml PBS yielding a final concentration of  $2 \times 10^{10}$  cells per ml. Seven ml of each of the inactivated cells was mixed with 7 ml of MPL + TDM adjuvant (Sigma Corp.) yielding a final  
30 concentration of  $1.0 \times 10^{10}$  cells per ml.

Whole cell bacterin preparations of the other eight top clinical isolates (*P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106, and *P. endodontalis* B114) or other pigmented anaerobic bacteria can be prepared in an identical fashion.

#### **HOMOLOGOUS VACCINE EFFICACY**

35 In homologous vaccine efficacy studies, mice were immunized with two injections of 0.2 ml each of the above mentioned inactivated *P. gulae* B43 cells in MPL + TDM adjuvant



three weeks apart. The mice were infected as previously described with *P. gulae* B43 two weeks following the booster immunization. Forty-two days following the infection, the mice were sacrificed and processed as previously described. Table 5 shows the numerical results of bone loss measurements.

5      **Table 5.** Mouse homologous vaccine efficacy study results.

Group	Vaccinogen	Challenge	Mean bone loss	Std. Dev.	SEM	Net bone loss	% bone loss (a)	% bone loss (b)
A	PBS with RIBI MPL+TDM adjuvant	None	0.0686	0.00862	0.00216	0	NA (c)	NA
B	PBS with RIBI MPL+TDM adjuvant	Pg 53977	0.112	0.0107	0.00266	0.0434	100	NA
C	PBS with RIBI MPL+TDM adjuvant	Pg B43	0.093	0.0188	0.00471	0.0244	NA	100
D	Formalin inactivated <i>P.</i> <i>gingivalis</i> 53977 with Freund's adjuvant	Pg 53977	0.098	0.0146	0.00364	0.0294	67.7	NA
E	Formalin inactivated <i>P.</i> <i>gingivalis</i> 53977 with RIBI MPL+TDM adjuvant	Pg 53977	0.0932	0.0109	0.00271	0.0246	56.7	NA
F	Formalin inactivated <i>P.</i> <i>gulae</i> B43 with RIBI MPL+TDM adjuvant	Pg B43	0.082	0.0128	0.00319	0.0134	NA	54.9
G	BEI inactivated	Pg B43	0.107	0.0151	0.0039	0.0384	NA	157.4

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Group	Vaccinogen	Challenge	Mean bone loss	Std. Dev.	SEM	Net bone loss	% bone loss (a)	% bone loss (b)
<hr/>								
	<i>P. gulae</i> B43							
	with RIBI							
	MPL+TDM							
	adjuvant							
H	Heat	Pg B43	0.0845	0.0113	0.00281	0.0159	NA	65.2
	inactivated <i>P. gulae</i> B43							
	with RIBI							
	MPL+TDM							
	adjuvant							
I	aeration	Pg B43	0.0746	0.00691	0.00173	0.006	NA	24.6
	inactivated <i>P. gulae</i> B43							
	with RIBI							
	MPL+TDM							
	adjuvant							

(a) Percentage calculated based on group B as the positive control group.

(b) Percentage calculated based on group C as the positive control group.

(c) NA = Not applicable.

5

Figures 6, 7, and 8 graphically display these results. Figure 7 shows the percent bone loss for the control experiment. Vaccines containing formalin-inactivated *P. gingivalis* 53977 and either Freund's complete/incomplete or MPL + TDM adjuvants reduced the bone loss induced by infection with *P. gingivalis* 53977 by approximately 32% and 43%, respectively. Figure 8 shows the percent bone loss for the test experiment. Vaccines containing either formalin-, heat-, or air-inactivated *P. gulae* B43 and MPL + TDM adjuvant reduced the bone loss induced by infection with *P. gulae* B43 by approximately 45%, 35%, and 75%, respectively. Based on these data, it can be concluded that the formalin-, air-, and heat-inactivated *P. gulae* B43 vaccines were efficacious in their ability to reduce bone loss induced in this superinfection model. Extrapolating this data into the clinical setting, these three vaccines would likely be efficacious in the prophylactic prevention of periodontal disease and may well prove efficacious in the therapeutic treatment of periodontal disease.

### HETEROLOGOUS VACCINE EFFICACY STUDY

5 In heterologous vaccine efficacy studies, mice were immunized with two injections of 0.2 ml each of either formalin-inactivated *P. gulae* B43 or formalin-inactivated *P. salivosa* B104 and *P. denticanis* B106 cells in MPL + TDM adjuvant three weeks apart. The mice were infected as previously described with either *P. gulae* B43, *P. gulae* B69, *P. salivosa* B104, or *P. denticanis* B106 two weeks following the booster immunization. Forty-two days following the infection, the mice were sacrificed and processed as previously described. Table 6 shows the numerical results of bone loss measurements.

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Table 6. Mouse heterologous vaccine efficacy study results.

Group	Vaccinogen	Inactivation method	Challenge	Mean	Std. Dev.	SEM	Net	% bone loss <sup>a</sup>	% bone loss <sup>b</sup>	% bone loss <sup>c</sup>	% bone loss <sup>d</sup>
A	PBS	NA	None	0.088	0.0112	0.00299	0	0	0	0	0
B	PBS	NA	<i>P. gulae</i> B43	0.101	0.0103	0.00266	0.013	100	NA <sup>e</sup>	NA	NA
C	PBS	NA	<i>P. gulae</i> B69	0.115	0.0112	0.00289	0.027	NA	100	NA	NA
D	PBS	NA	<i>P. salivosa</i> B104	0.101	0.0132	0.00352	0.013	NA	NA	100	NA
E	PBS	NA	<i>P. pfizerii</i> B106	0.0994	0.0135	0.0035	0.0114	NA	NA	NA	100
F	<i>P. gulae</i> B43	Formalin	<i>P. gulae</i> B43	0.0901	0.016	0.00412	0.0021	16.15	NA	NA	NA
G	<i>P. gulae</i> B43	Formalin	<i>P. gulae</i> B69	0.104	0.0166	0.00443	0.016	NA	59.26	NA	NA
H	<i>P. gulae</i> B43	Formalin	<i>P. salivosa</i> B104	0.0926	0.0119	0.00319	0.0046	NA	NA	35.38	NA
I	<i>P. gulae</i> B43	Formalin	<i>P. pfizerii</i> B106	0.102	0.0124	0.00333	0.014	NA	NA	NA	122.8
J	<i>P. salivosa</i> B104/ <i>P. denticanis</i> B106	Formalin	<i>P. gulae</i> B69	0.102	0.0124	0.00333	0.014	NA	51.85	NA	NA

<sup>a</sup> Percentage bone loss is calculated for the *P. gulae* B43 infected mice.<sup>b</sup> Percentage bone loss is calculated for the *P. gulae* B69 infected mice.<sup>c</sup> Percentage bone loss is calculated for the *P. salivosa* B104 infected mice.<sup>d</sup> Percentage bone loss is calculated for the *P. denticanis* B106 infected mice.<sup>e</sup> NA, not applicable.

Figures 9, 10, 11, 12, and 13 graphically display these results. Figure 9 shows the net bone loss for these experiments. Figure 10 shows the percent bone loss for the *P. gulae* B43 infected groups. Formalin-inactivated *P. gulae* B43 and MPL + TDM adjuvant reduced the bone loss induced by infection with *P. gulae* B43 by approximately 84%. Figure 11 shows the percent bone loss for the *P. gulae* B69 infected groups. The formalin-inactivated *P. gulae* B43 and formalin-inactivated *P. salivosa* B104/*P. denticanis* B106 vaccines containing MPL + TDM adjuvant reduced the bone loss induced by infection with *P. gulae* B69 by approximately 40% and 49%, respectively. Figure 12 shows the percent bone loss for the *P. salivosa* B104 infected groups. Formalin-inactivated *P. gulae* B43 and MPL + TDM adjuvant reduced the bone loss induced by *P. salivosa* B104 by approximately 65%. Figure 13 shows the percent bone loss for the *P. denticanis* B106 infected groups. Formalin-inactivated *P. gulae* B43 with MPL + TDM adjuvant failed to cross protect against challenge with *P. denticanis* B106. Based on these data, it can be concluded that the formalin-inactivated *P. gulae* B43 vaccine adjuvanted with MPL + TDM was capable of providing protection not only from homologous challenge, but also from heterologous challenge with *P. gulae* B69. Moreover, protection was observed between two *Porphyromonas* species as the *P. gulae* B43 vaccine protected against *P. salivosa* B104 challenge. Extrapolating this data into the clinical setting, a multi-valent vaccine would likely be efficacious in the prophylactic prevention of periodontal disease and may well prove efficacious in the therapeutic treatment of periodontal disease.

#### Recombinant FimA and OprF mouse serological study

In subunit vaccine serology studies, mice were immunized with two injections of 0.2 ml each of either recombinantly expressed, purified *P. gulae* B43 FimA or recombinantly expressed, purified *P. gulae* B43 OprF in QuilA/Cholesterol adjuvant three weeks apart. The mice were bled prior to the first vaccination and two weeks following the booster immunization. Table 7 shows the numerical results while figures 14 and 15 show the results graphically.

**Table 7.** Mouse subunit vaccine serology study.

Group	Vaccinogen	rFimA ELISA		rOprF ELISA	
		Pre-vaccination	Post-vaccination	Pre-vaccination	Post-vaccination
A	Saline	50	50	50	50
B	rFimA + QAC	50	138889	NA	NA
C	rOprF + QAC	NA	NA	50	118

- 5 Throughout this application, various patent and scientific publications, including United States patents, are referenced by author and year and patents by number. The disclosures of these publications and patents are hereby incorporated by reference in their entireties into this application in order to more fully describe the state of the art to which this invention pertains.

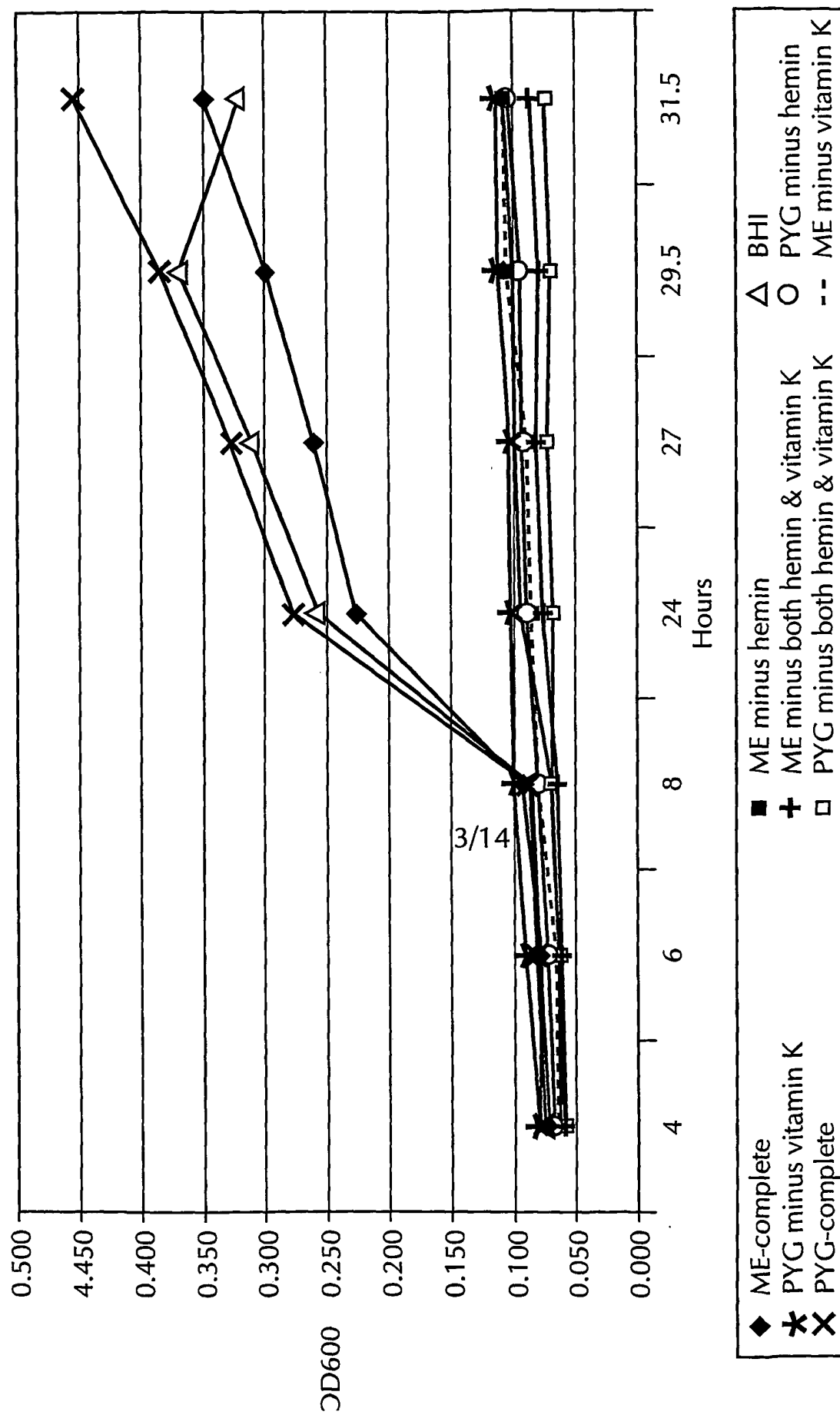
### CLAIMS

1. An isolated pigmented anaerobic bacteria having a 16S rRNA DNA sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 94, provided that the bacteria is not a strain of *Porphyromonas gingivalis* designated as dog  
5 20B.
2. An isolated pigmented anaerobic bacteria which causes, either directly or in combination with other pathogenic agents, periodontal disease in companion animals, wherein the bacteria can be used to prepare a vaccine for treating or preventing periodontal disease in companion animals, wherein the vaccine comprises an immunologically effective  
10 amount of the bacteria which has been inactivated or attenuated, provided that the bacteria is not a strain of *P. gulae* sp. nov. designated ATCC 51700.
3. The bacteria according to claim 2 having a 16S rRNA DNA sequence at least about 95% homologous to any of the sequences depicted in SEQ ID NOS: 86 to 94.
- 4 An isolated polynucleotide molecule comprising a nucleotide sequence  
15 isolated from a bacteria selected from the group consisting of a bacterium having the identifying characteristics of *Porphyromonas gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *P. denticanis* B106 and *P. endodontalis* B114 provided that the bacteria is not a strain of *P. gulae* sp. nov. designated ATCC 51700.
- 20 5. The isolated polynucleotide according to claim 4 wherein the polynucleotide encodes for a polypeptide.
6. The isolated polynucleotide according to claim 4 wherein, the polynucleotide encodes ribosomal RNA or transfer RNA.
7. An isolated polynucleotide molecule comprising any of the nucleotide  
25 sequences selected from the group consisting of SEQ ID NOS: 86 to 94 and homologues having at least 95% homology thereto, provided that the nucleotide sequence is not the 16S rRNA DNA from bacteria *P. gulae* sp. nov. designated ATCC 51700.
8. An isolated polynucleotide molecule comprising any of the nucleotide sequences depicted in SEQ ID NOS: 95 to 102 and 111-119 or fragments or variants thereof,  
30 which sequence encodes a polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, or complements thereto.
9. isolated polynucleotide molecule comprising a nucleotide sequence which hybridizes under conditions of high stringency to any of the sequences depicted in SEQ ID NOS: 95 to 102 and 111-119, or complements thereto.
- 35 10. A recombinant expression vector comprising a polynucleotide selected from the group consisting of any of the nucleotide sequences SEQ ID NOS: 95 to 102 and 111 to 119, fragments or variants thereof, operably linked to a promoter sequence.

11. A plasmid comprising a polynucleotide selected from the group consisting of any of the nucleotide sequences SEQ ID NOS: 95 to 102 and 111 to 119, fragments or variants thereof, operably linked to a promoter sequence.
12. A host cell comprising the isolated polynucleotide sequence according to claim 4,  
5 7, or 8.
13. A method for the production of recombinant FimA, OprF, selected from any of the sequences depicted in SEQ ID NOS: 103 to 110 or 120 to 128, or fragments or variants thereof, said method comprising (1) growing the cells of claim 36 under conditions in which a polypeptide comprising FimA, or OprF, or fragments or variants thereof is expressed, and (2)  
10 recovering said polypeptide.
14. An isolated polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals comprising an amino acid sequence depicted in SEQ ID NOS: 103 to 110 and 120 to 128.
15. A recombinantly expressed polypeptide, which polypeptide is selected from  
15 the group consisting of FimA (SEQ ID NOS: 103 to 110) and OprF (SEQ ID NOS: 120 to 128).

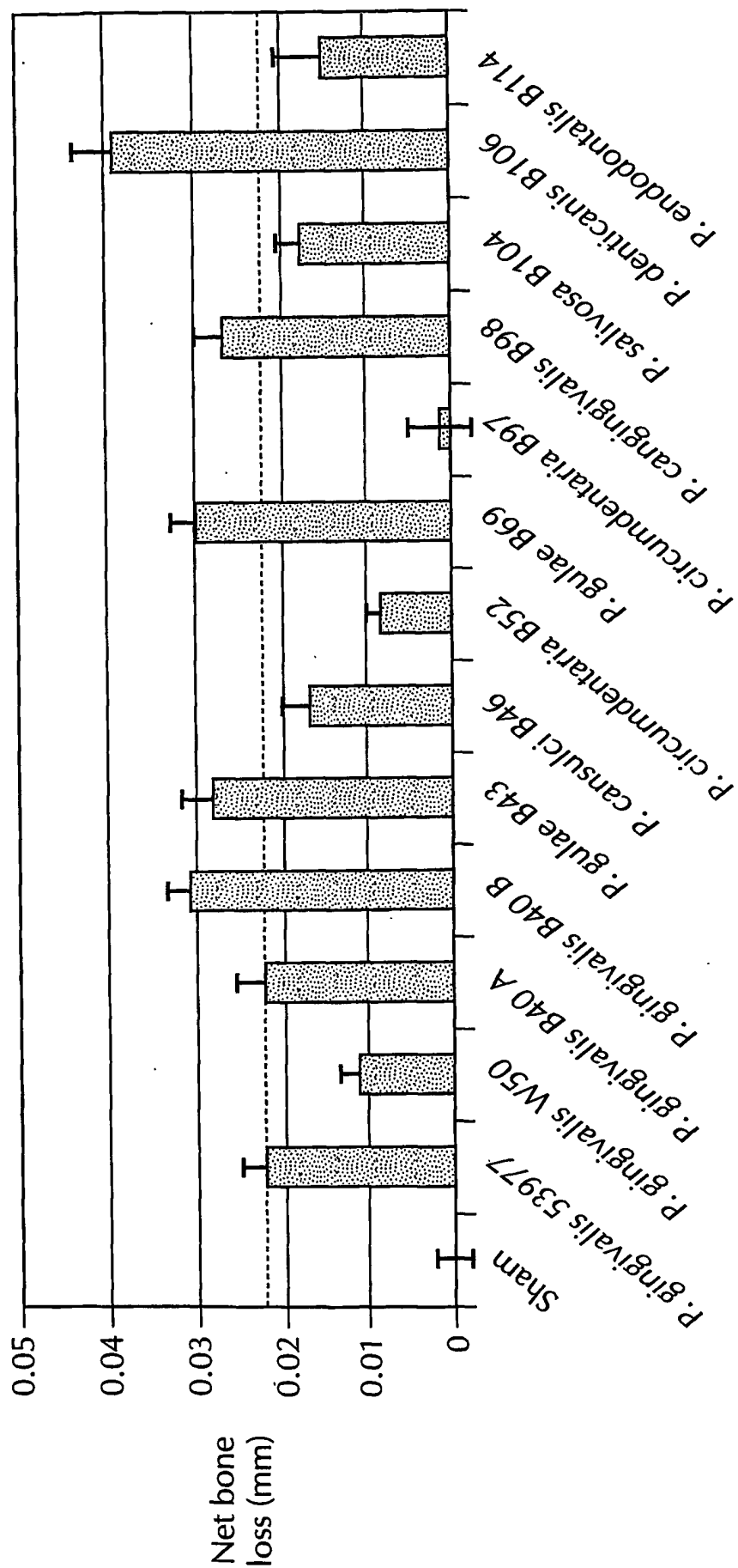


**FIG. 1**



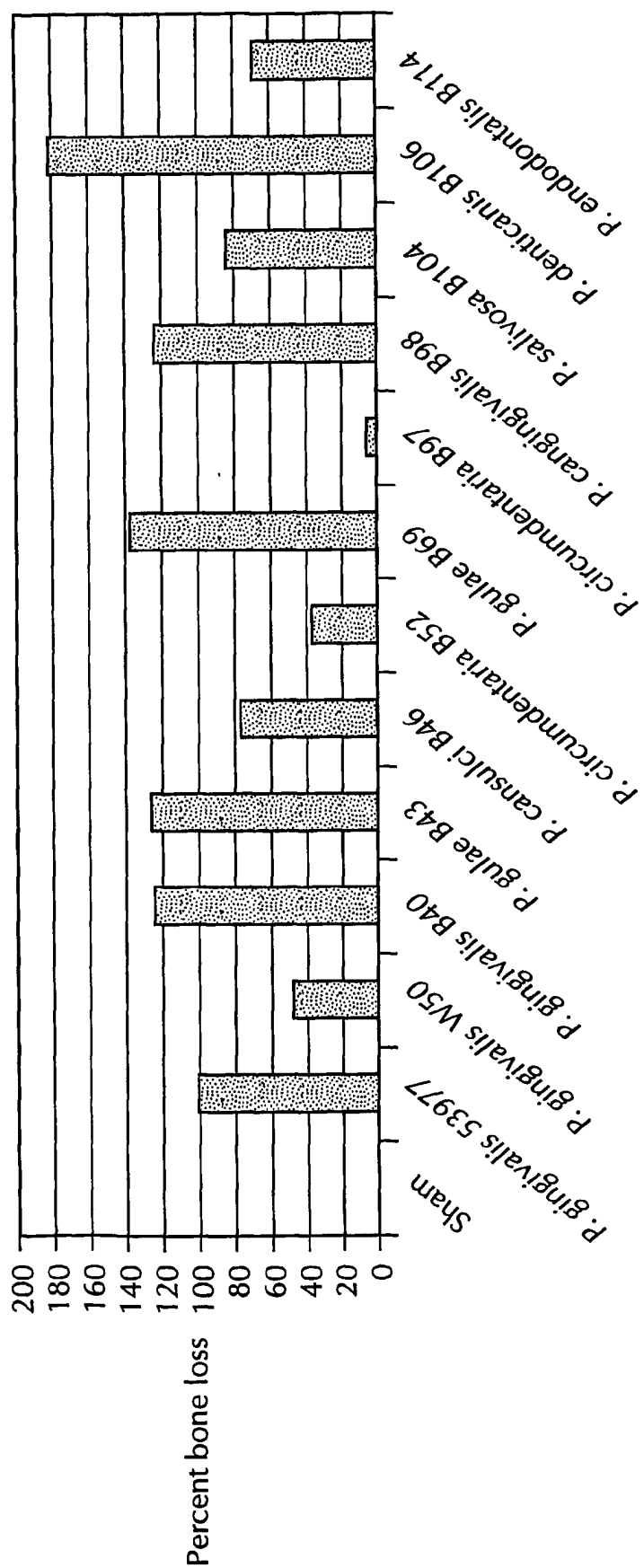
2/14

FIG. 2



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FIG. 3



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FIG. 4B

5  $\lambda$  Insol  
1  $\lambda$  Insol  
5  $\lambda$  Sol  
1  $\lambda$  Sol  
Std  
Ind

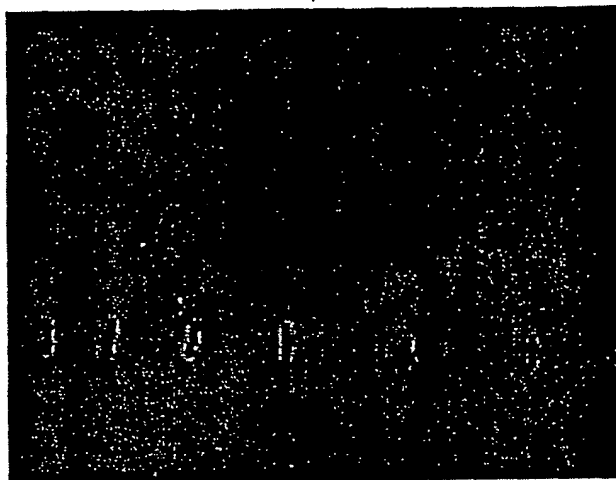
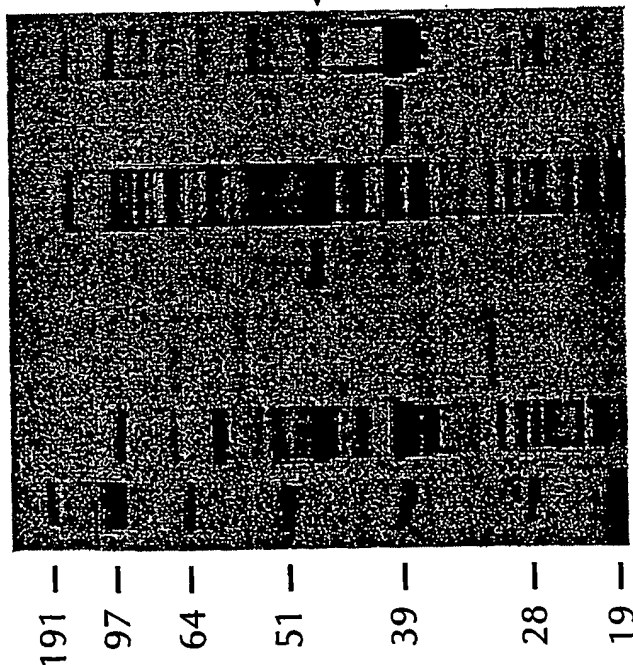


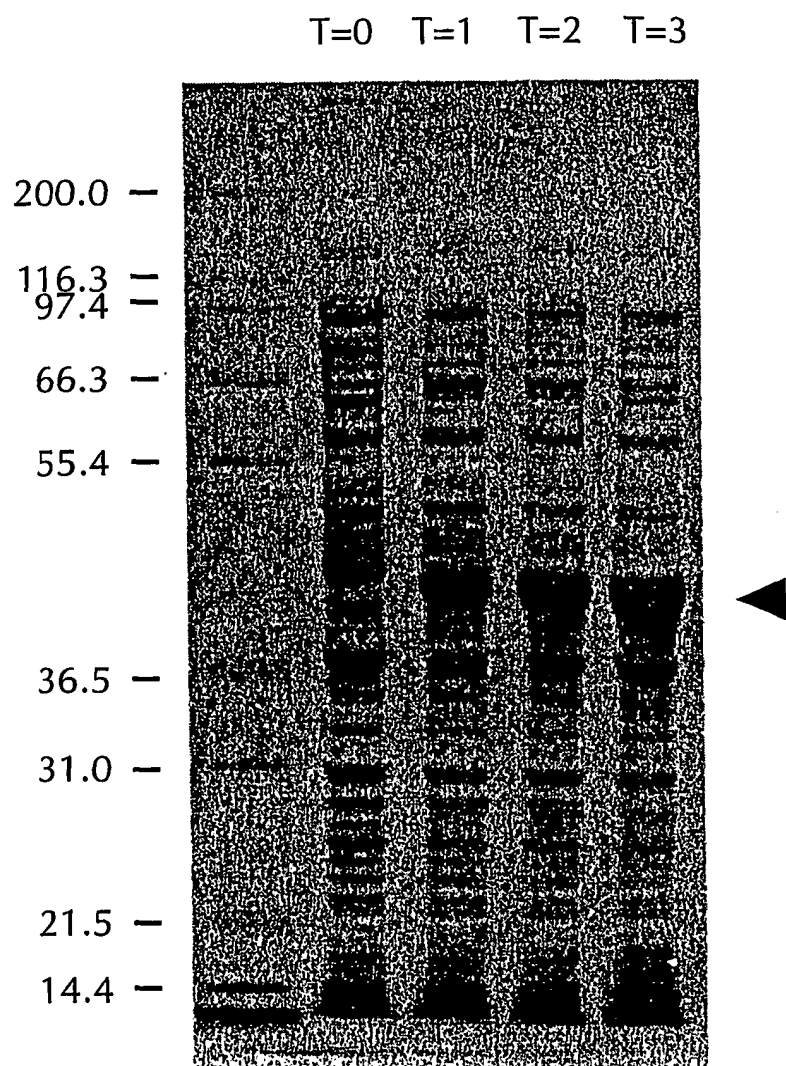
FIG. 4A

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5  $\lambda$  Sol  
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Std  
Ind  
Std



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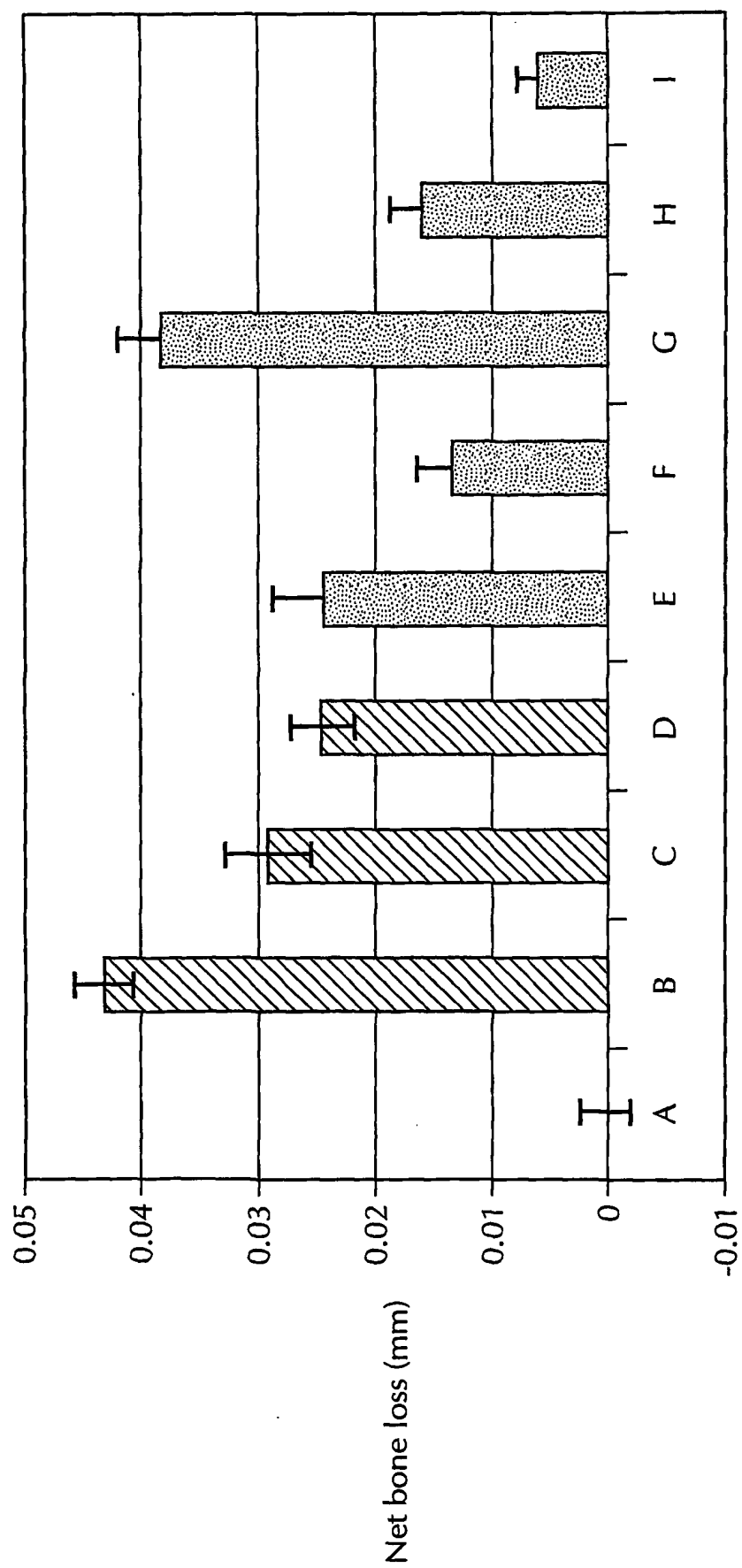
5/14

**FIG. 5**

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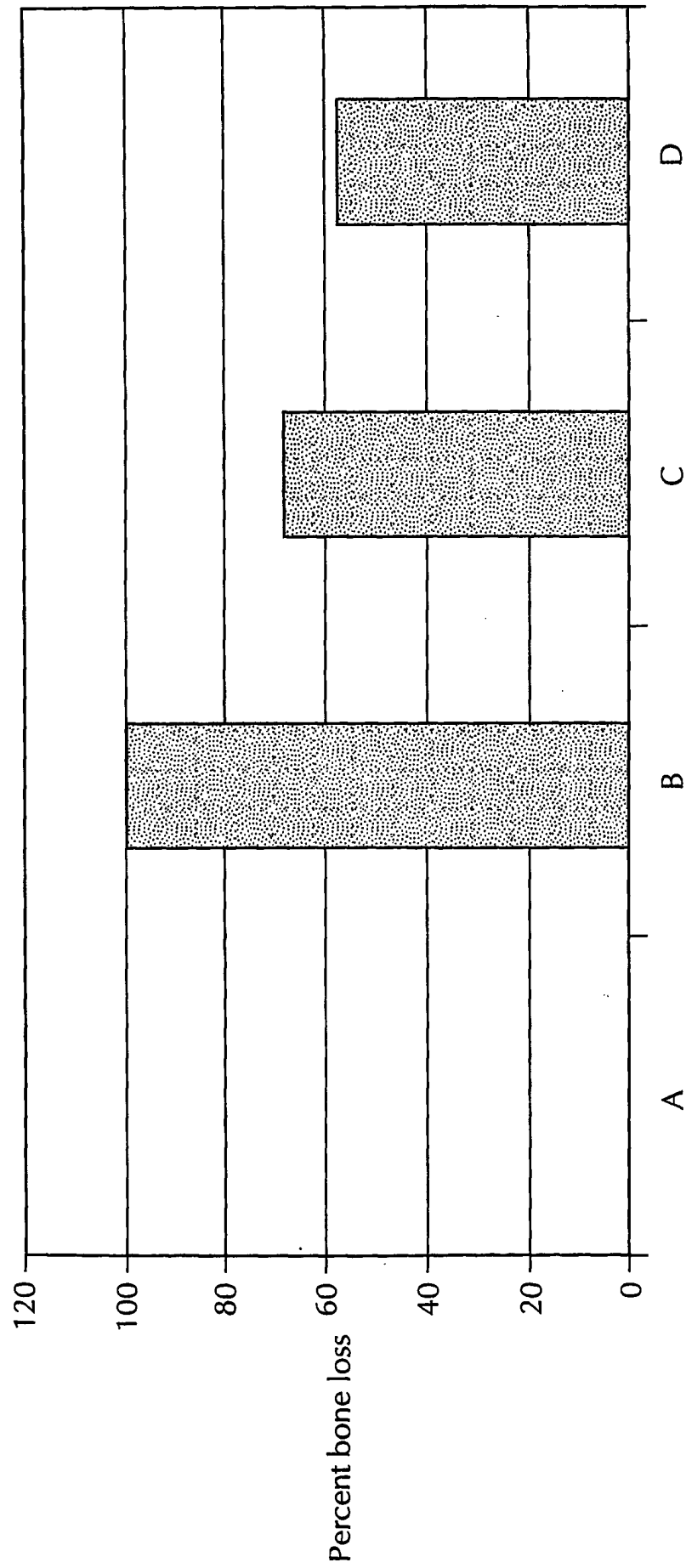
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FIG. 6



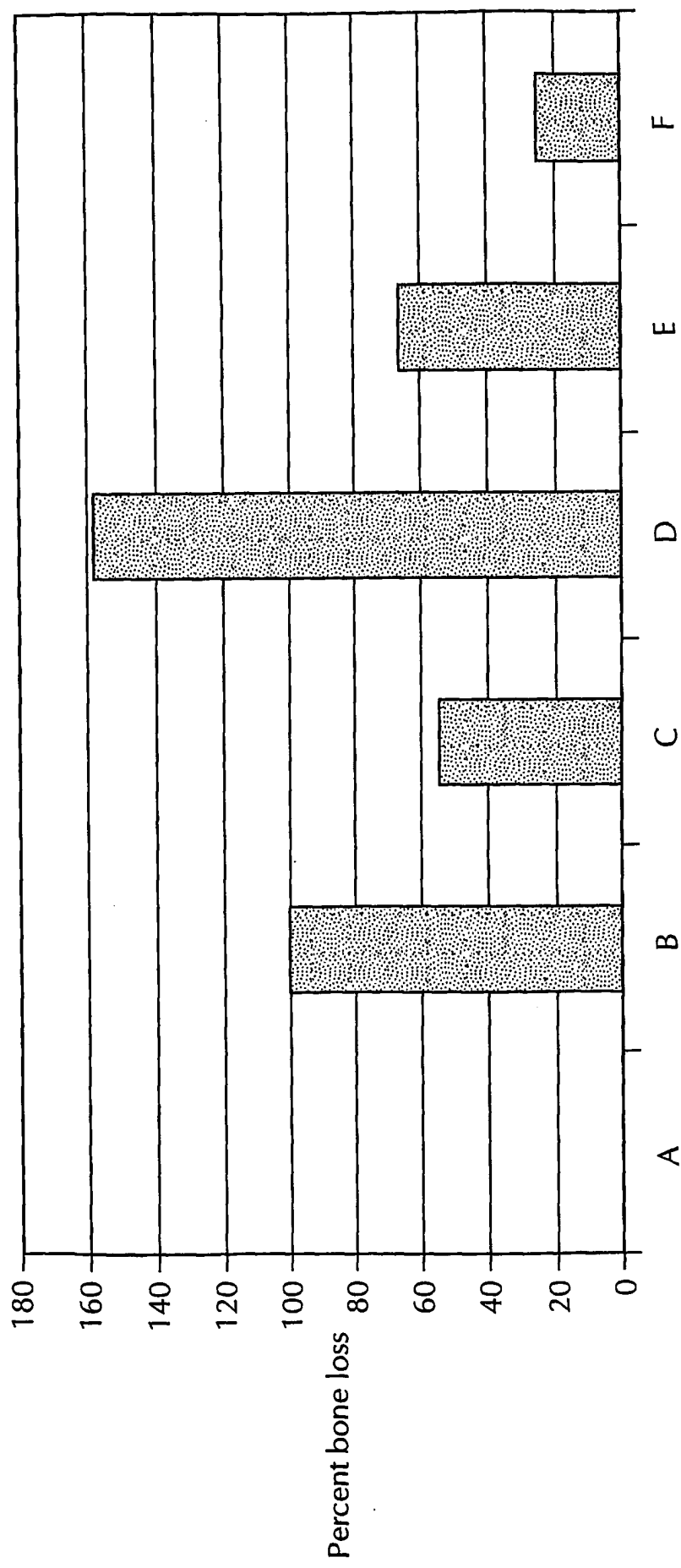
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FIG. 7



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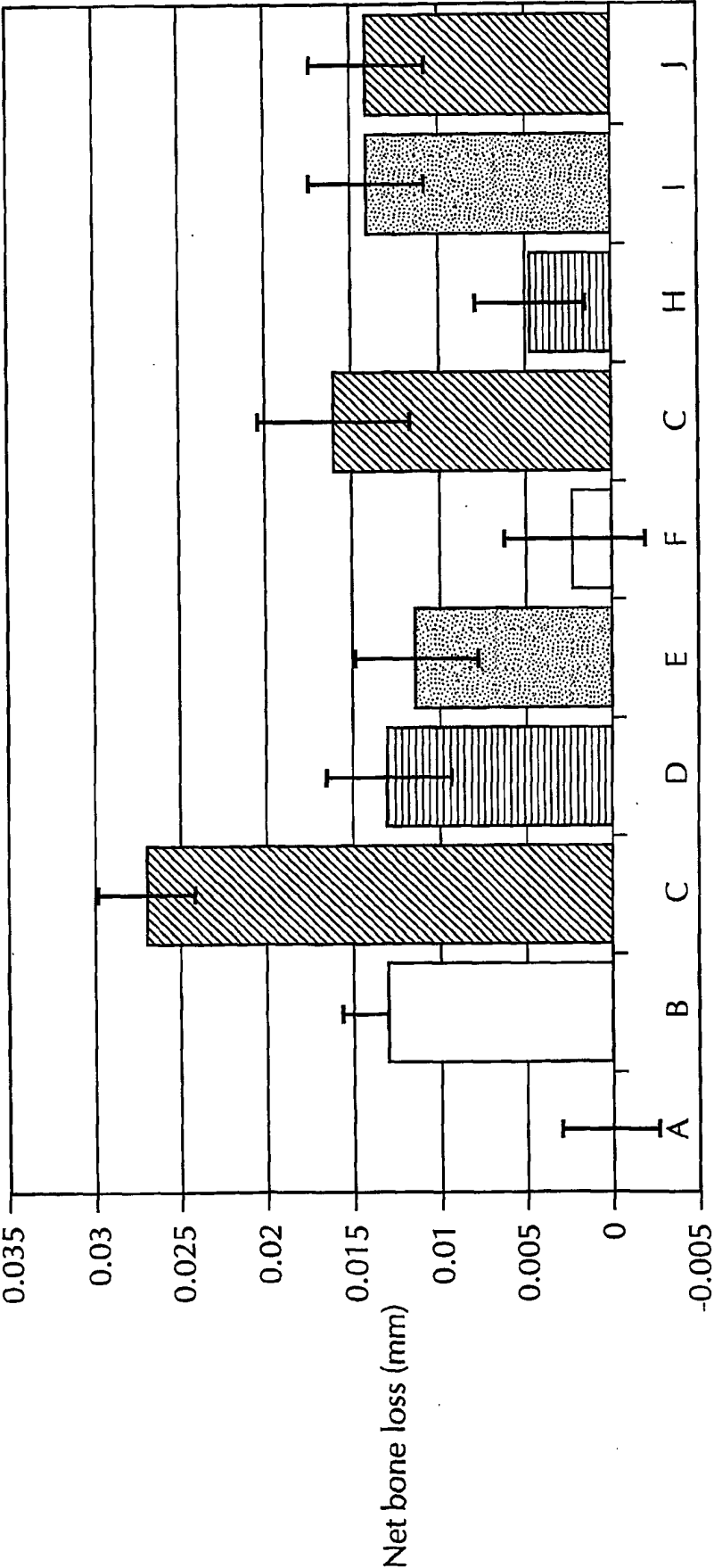
FIG. 8





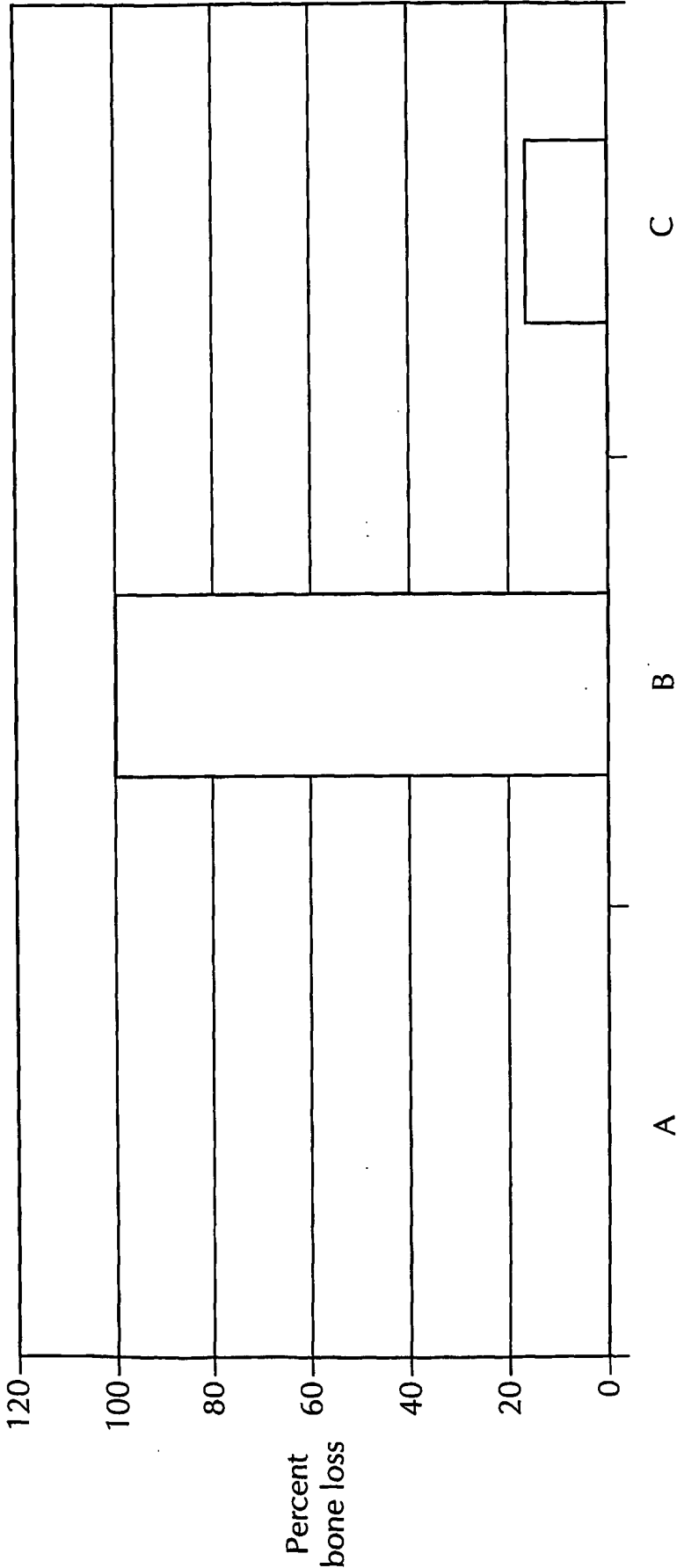
9/14

FIG. 9



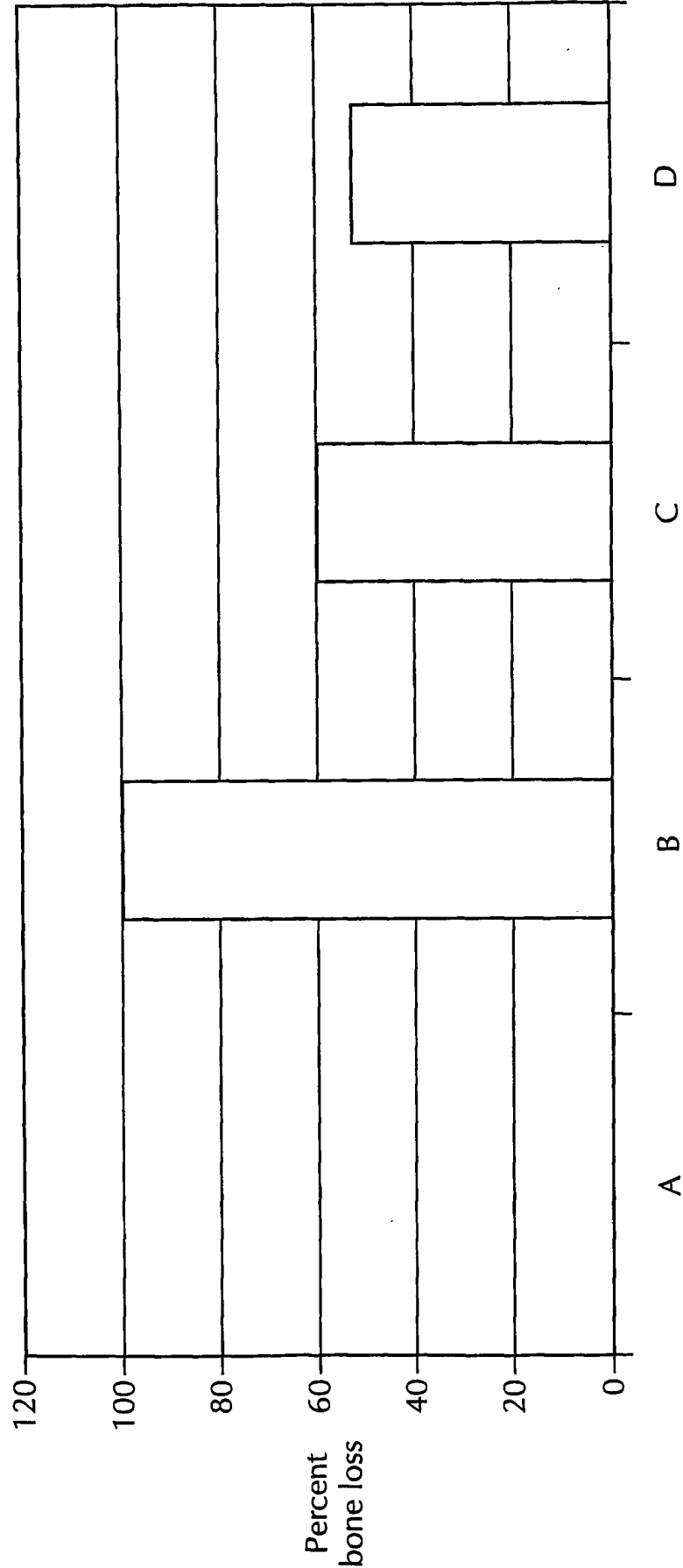
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FIG. 10



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FIG. 11



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FIG. 12

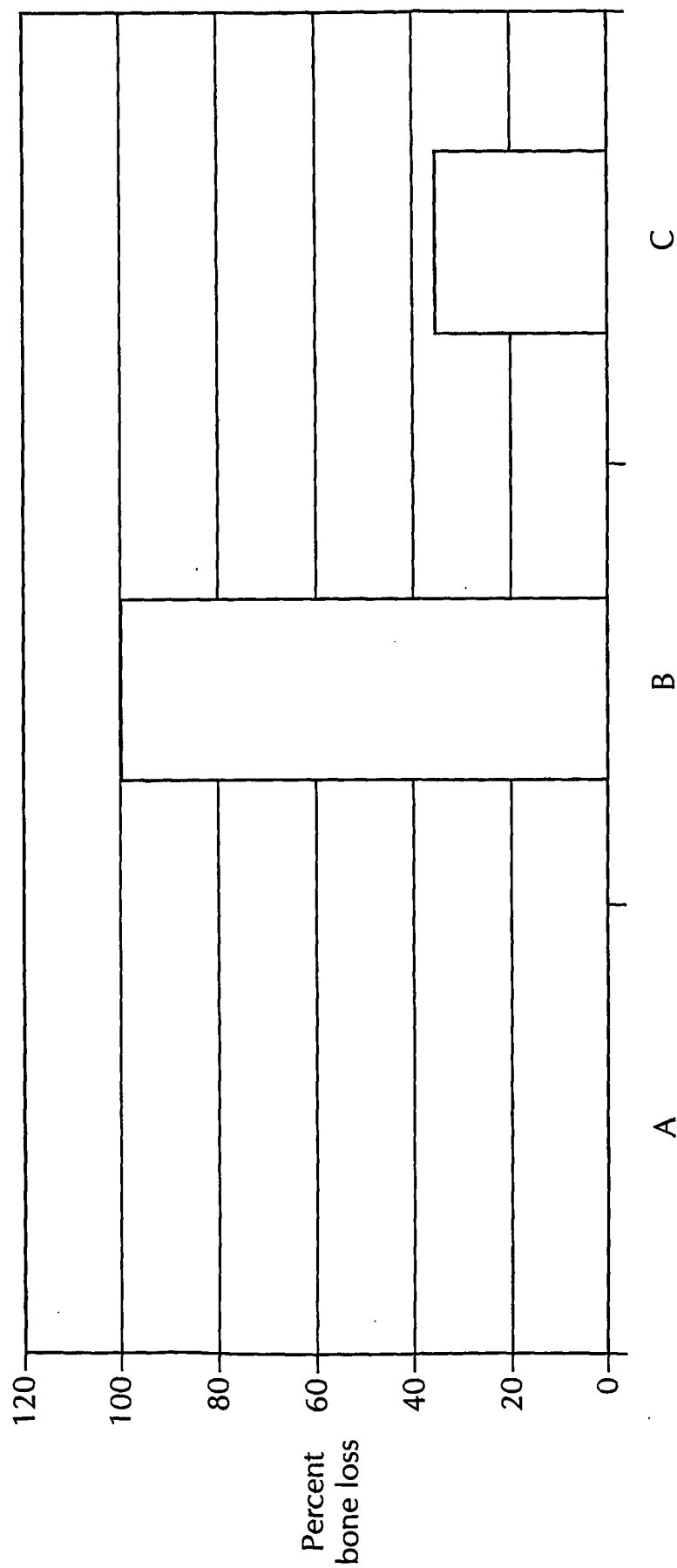
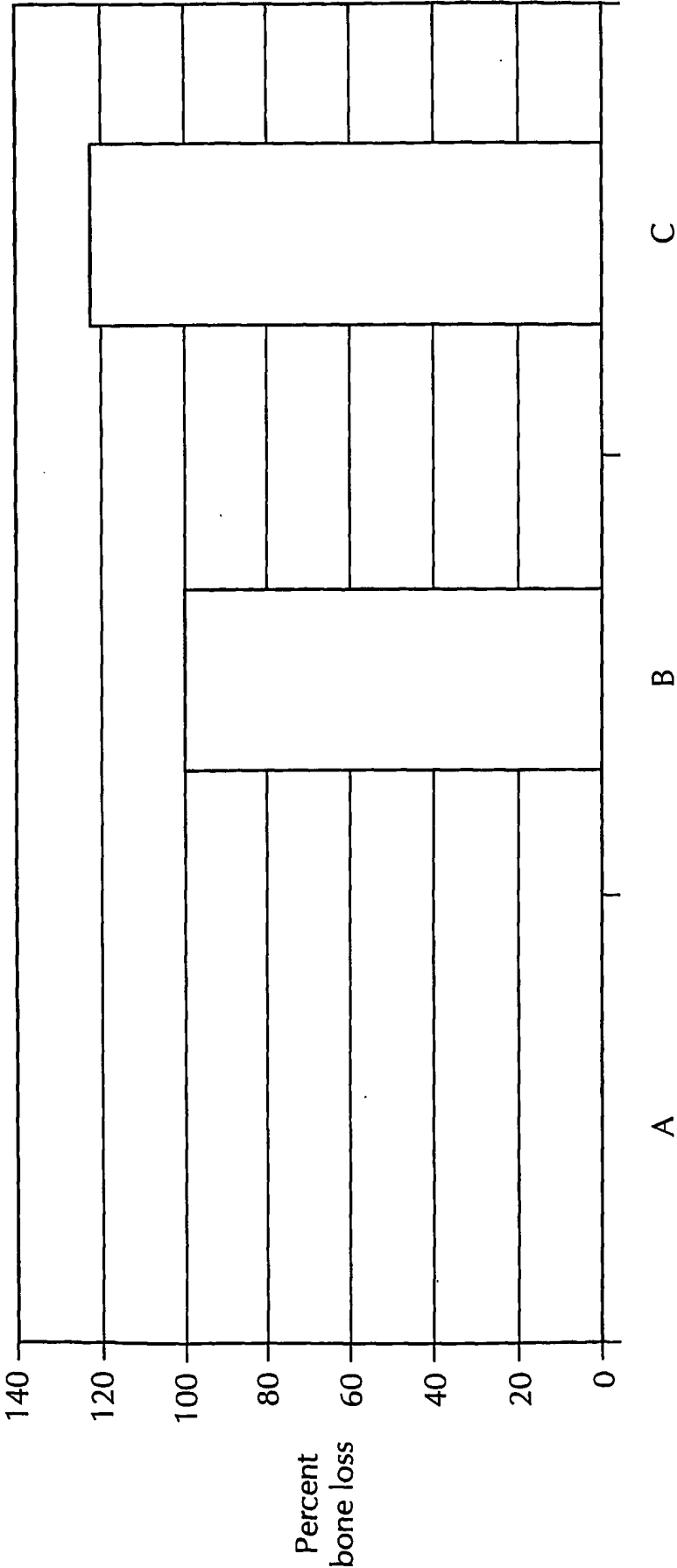
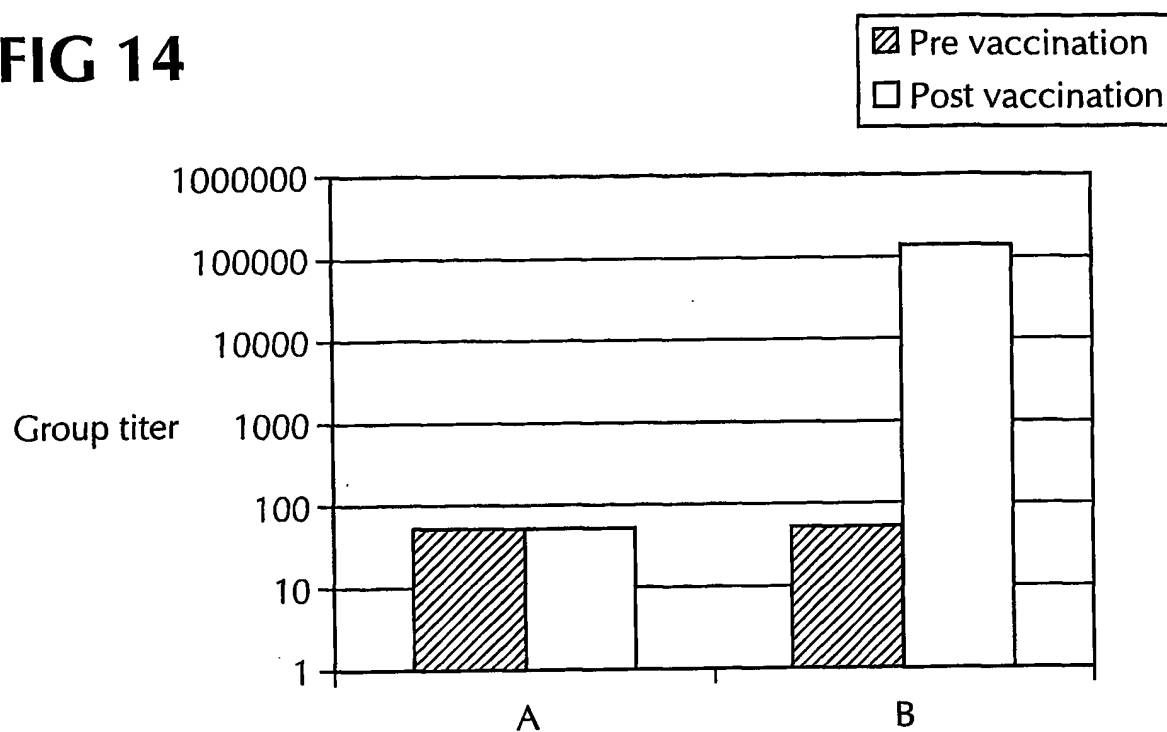
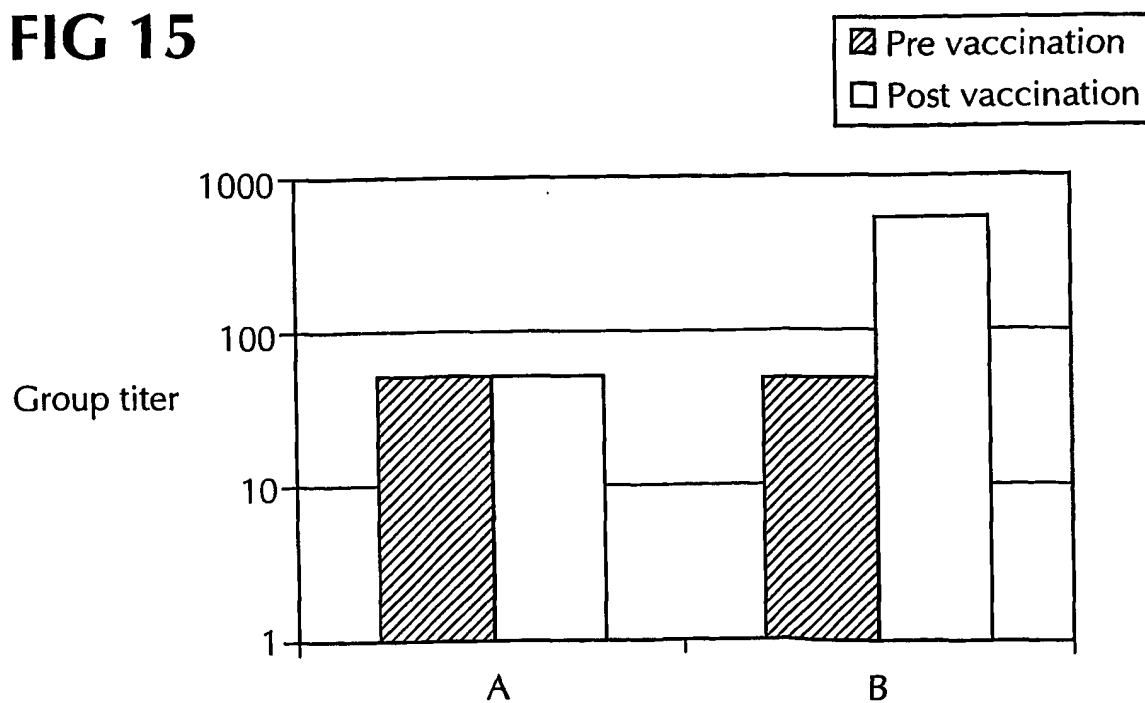


FIG. 13



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**FIG 14****FIG 15**

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<210> 7

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<223> Description of Artificial Sequence:D0068

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<210> 8

<211> 23

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<223> Description of Artificial Sequence:D0078

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gcgacgctat atgcaagaca atc

23

<210> 9

<211> 33

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<223> Description of Artificial Sequence:D0097

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33

<210> 10

<211> 34

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<223> Description of Artificial Sequence:D0098

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<210> 11

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<400> 11

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<210> 12

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<223> Description of Artificial Sequence:PFZ185-AP2

<400> 12

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<210> 13

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<210> 14

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<210> 15

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<223> Description of Artificial Sequence:PFZ186-AP3

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<210> 18

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<223> Description of Artificial Sequence:PFZ186-AP4

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<210> 19

<211> 20

<212> DNA

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<223> Description of Artificial Sequence:PFZ186-AP5

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<210> 20

<211> 20

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<210> 21

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<210> 22

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<223> Description of Artificial Sequence:PFZ187-AP3

<400> 22

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<210> 23

<211> 20

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<223> Description of Artificial Sequence:PFZ187-AP4

<400> 23

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<210> 24

<211> 20

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<223> Description of Artificial Sequence:PFZ187-AP5

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<210> 25

<211> 20

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<223> Description of Artificial Sequence:PFZ187-AP6

<400> 25

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<210> 26

<211> 20

<212> DNA

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<223> Description of Artificial Sequence:PFZ187-AP7

<400> 26

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<210> 27

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<223> Description of Artificial Sequence:PFZ187-AP8

<400> 27

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<210> 28

<211> 20

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<223> Description of Artificial Sequence:PFZ187-AP9

<400> 28

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<210> 29

<211> 20

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<223> Description of Artificial Sequence:PFZ187-AP11

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<210> 30

<211> 20

<212> DNA

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<223> Description of Artificial Sequence:PFZ187-AP12

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<210> 31

<211> 20

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<210> 32  
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<210> 33  
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<210> 34  
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<210> 35  
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<400> 35

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<210> 36

<211> 20

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<210> 37

<211> 20

<212> DNA

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<223> Description of Artificial Sequence:PFZ188-AP1

<400> 37

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<210> 38

<211> 20

<212> DNA

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<400> 38

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<210> 39

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<223> Description of Artificial Sequence:PFZ188-AP3

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<210> 40

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<400> 40

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<210> 41

<211> 20

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<223> Description of Artificial Sequence:PFZ188-AP5

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<210> 42

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<223> Description of Artificial Sequence:PFZ188-AP6

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<210> 43

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<223> Description of Artificial Sequence:D0086

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<210> 44

<211> 25

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<210> 45

<211> 27

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27

<210> 46

<211> 20

<212> DNA

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<223> Description of Artificial Sequence:PFZ209-AP1

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<210> 47

<211> 20

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<223> Description of Artificial Sequence:PFZ209-AP2

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<210> 48

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<223> Description of Artificial Sequence:PFZ209-AP3

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<210> 49

<211> 20

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<223> Description of Artificial Sequence:PFZ209-AP4

<400> 49

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<210> 50

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<223> Description of Artificial Sequence:PFZ210-AP1

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<210> 51

<211> 20

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<223> Description of Artificial Sequence:PFZ210-AP2

<400> 51

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<210> 52

<211> 20

<212> DNA

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<223> Description of Artificial Sequence:PFZ210-AP3

<400> 52

cgcttggaga gttcttcgac

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<210> 53

<211> 20

<212> DNA

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<223> Description of Artificial Sequence:PFZ210-AP4

<400> 53

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<210> 54

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<223> Description of Artificial Sequence:PFZ211-AP1

<400> 54

aactacttca agccctacag

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<210> 55

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<223> Description of Artificial Sequence:PFZ211-AP2

<400> 55

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<210> 56

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<212> DNA

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<400> 56

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<210> 57

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<400> 57

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<210> 58

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<223> Description of Artificial Sequence:PFZ211-AP5

<400> 58

atacgctcta cacgaggctc

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<210> 59

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<223> Description of Artificial Sequence:PFZ212-AP1

<400> 59

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<210> 60

<211> 20

<212> DNA

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<223> Description of Artificial Sequence:PFZ212-AP2

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<210> 61

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<210> 62

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<223> Description of Artificial Sequence:PFZ212-AP4

<400> 62

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<210> 63

<211> 20

<212> DNA

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<400> 63

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<210> 64

<211> 20

<212> DNA

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<223> Description of Artificial Sequence:PFZ213-AP2

<400> 64

tttgtgttg taaccaacac

20

<210> 65

<211> 20

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<223> Description of Artificial Sequence:PFZ213-AP3

<400> 65

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20

<210> 66

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<223> Description of Artificial Sequence:PFZ213-AP4

<400> 66

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<210> 67

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<223> Description of Artificial Sequence:PFZ213-AP5

<400> 67

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<210> 68

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<400> 68

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<210> 69

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<212> DNA

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<400> 69

ccaacaccga accaaggcac

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<210> 70

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<223> Description of Artificial Sequence:PFZ214-AP3

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<210> 71

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<223> Description of Artificial Sequence:PFZ214-AP4

<400> 71



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<210> 72

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<223> Description of Artificial Sequence:PFZ215-AP1

<400> 72

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20

<210> 73

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<400> 73

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<210> 74

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<400> 74

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<210> 75

<211> 20

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<400> 75

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<210> 76

<211> 27

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<223> Description of Artificial Sequence:KWK-Pg-06

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atgcaggaaa atactgtacc ggcaacg

27

<210> 77

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29

<210> 78

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31

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<210> 80

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21

<210> 81

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<223> Description of Artificial Sequence:KWK-Ps-04b

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37

<210> 82

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<223> Description of Artificial Sequence:KWK-Ps-05b

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34

<210> 83

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39

<210> 84

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<223> Description of Artificial Sequence:D122

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25

<210> 85

<211> 25

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<223> Description of Artificial Sequence:D123

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25

<210> 86

<211> 572

<212> DNA

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<223> Description of Artificial Sequence:P. guiae B43  
16S rRNA polynucleotide sequence

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gcgttaagta atccacctgg ggagtacgcc ggcaacggtg aaactcaaag gaattgacgg 120  
gggcccgcac aagcggagga acatgtggtt taattcgatg atacgcgagg aaccttacct 180  
gggattgaaa tgtagacgac ggatggtgaa agccgtcttc ccttcggggc gtctatgtag 240  
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gcaaccacaca tcggtagttg ctaacagggt tagctgagga ctctaccgag actgccgtcg 360  
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acacgtgtta caatgggagg gacaaagggc agtaccggg cgaccgggtg cgaatctcga 480  
aacccttccc cagttcggat cggagtctgc aactcgactc cgtgaagctg gattcgctag 540  
taatcgcgca tcagccatgg cgcggtgaat ac 572

<210> 87  
 <211> 571  
 <212> DNA  
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<220>

<223> Description of Artificial Sequence:P. cansulci B46  
 16S rRNA polynucleotide sequence

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 ggcccgacaca agcggaggaa catgtggttt aattcgatga tacgcgagga acctacccg 180  
 ggattgaaat atagatgaca ggcagcgaga gttgttatcc ctccggggca tctatgtagg 240  
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 caaccacat tattagttac taacagggtta agctgaggac tctaataaga ctgccggcgt 360  
 aagccgtgag gaaggtgtgg atgacgtcaa atcagcacgg cccttacatc cggggcgaca 420  
 cacgtgttac aatggtaggg acaaagggca gctaccgggc gaccggatgc gaatctccaa 480  
 accctatccc agttcggatc ggagtcgtga actcgactct gtgaagctgg attcgctagt 540  
 aatcgcgcat cagccatggc gcggtgaata c 571

<210> 88  
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 <212> DNA  
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<220>

<223> Description of Artificial Sequence:P.  
 circumdentaria B52 16S rRNA polynucleotide  
 sequence

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 gccgcacaa gcggaggaa atgtggttta attcgatgat acgcgaggaa ccttacctgg 180  
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 gcaaccgcg ttgatagtta ctaacagata aagctgagga ctctatcgag acagccgtcg 360  
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<210> 89  
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&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:P. guiae B69

16S rRNA polynucleotide sequence

&lt;400&gt; 89

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cacgcagtaa acgatgatta ctaggagttt gcgatatacc gataagcttc cacagcgaaa 60
gcgttaagta atccacctgg ggagtagccc ggcaacggtg aaactcaaag gaattgacgg 120
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gggattgaaa tgtagatgac agatggtgaa agccgtcttc ccttcggggc gtctatgtag 240
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gcaaccata tcggtagttg ctaacaggtc aagctgagga ctctaccgag actgccgtcg 360
taaggcgaga ggaagggtgt gatgacgtca aatcagcacg gcccttacat ccggggcgac 420
acacgtgtta caatgggagg gacaaagggc agctaccggg cgaccggatg cgaatctcga 480
aacccttccc cagttcggat cggagtctgc aactcgactc cgtgaagctg gattcgctag 540
taatcgcgca tcagccatgg cgcggtgaat acc 573

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&lt;210&gt; 90

&lt;211&gt; 572

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:P.

circumdentaria B97 16S rRNA polynucleotide  
sequence

&lt;400&gt; 90

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cacgctgtaa acgatgaata ctagatTTTT gcgatataca gtaagagtct aagcgaaagc 60
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gcccgcacaa gcggaggaac atgtggttta attcgatgat acgcgaggaa ccttacctgg 180
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gcaaccgcg tcgatagtta ctaacaggta atgctgagga ctctatcgag acagccgtcg 360
taagacgaga ggaagggggc gatgacgtca aatcagcacg gcccttacat ccagggcgac 420
acacgtgtta caatggcaag gacaaagga agccacatag cgatatggag cagatcctca 480
aaccttgtcc cagttcggat cggagtctgc aactcgactc cgtgaagctg gattcgctag 540
taatcgcgca tcagccatgg cgcggtgaat ac 572

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&lt;210&gt; 91

&lt;211&gt; 571

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:P.

canggingivalis B98 16S rRNA polynucleotide sequence

&lt;400&gt; 91

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cagtaaacga tgattactcg gagtatgcga tatatgggtat gctcccaagg gaaaccgata 60
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gcacaagcgg aggaacatgt ggtttaattc gatgatacgc gaggaacctt acccgggatt 180
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gcgcgaggaa ggtgtggatg acgtcaaatac agcacggccc ttacatccgg ggcgacacac 420
gtgtttacaat ggtagggaca aagggcagct acctggcgac aggatgcgaa tctccaaacc 480
ctatctcagt tcggatcgga gtctgcaact cgactccgtg aagctggatt cgctagtaat 540
gcgcgcatcag ccatggcgcg gtgaatacgt t 571

```

&lt;210&gt; 92

&lt;211&gt; 384

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:P. salivosa

B104 16S rRNA polynucleotide sequence

&lt;400&gt; 92

```

cagtaaacga tgataactgg gcgtatgcga tatacagtat gtccttgagc gaaagcgtaa 60
agttatccac ctggggagta cgccggcaac ggtgaaactc aaaggaattg acggggggccc 120
gcacaagcgg aggaacatgt ggtttaattc gatgatacgc gaggaacctt acccgggatt 180
gaaatthtagc ggactatgta tgaaagtaca taccctgtca caaggccgct aagtaggtgc 240
tgcatgggttgcgtcagctc gtgccgtgag gtgtcggctt aagtgccata acgagcgcaa 300
cccacgttgt cagttactat cgggttaaagc cgaggactct gacaagactg ccgtcgtaag 360
gcgcgaggaa ggtgtggatg acgt 384

```

&lt;210&gt; 93

&lt;211&gt; 571

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:P. denticanis

B106 16S rRNA polynucleotide sequence

&lt;400&gt; 93

```

cacgccgtaa acgatgctca ccggctctat gcgataagac agtatggggc taatagaaat 60
aattaagtga gccacctggg gaggtagctc gcaacgatga aactcaaagg aattgacggg 120

```

```

ggcccgacac agcggaggaa catgtggttt aattcgatga tacgcgagga accttaccg 180
ggtttaaagt tatgttgcat tatgtagaaa tacgtatttt cttcggaact gcatacaagg 240
tgctgcatgg ttgtcgtcag ctctgtccgt gaggtgtcgg gttaagtccc ataacgagcg 300
caacccttat gattagttgc taacggttca agccgagcac tctattcaca ctgccaccgt 360
aaggtgagag gaaggagggg atgatgtcaa atcagcacgg cccttatatc cggggctaca 420
cacgtgttac aatggtcggg acagcggggt gcatttacgt gagtaacagc taatcccaaa 480
aatcggcttc agttcggatt ggagtctgca actcgactcc atgaagttgg attcgctagt 540
aatcgacacat cagccatggg gcggtgaata c 571

```

<210> 94

<211> 571

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: *P. endodontalis*  
B114 16S rRNA polynucleotide sequence

<400> 94

```

caccgcagta aacgatgaat actagatctt tgcgatatac ggtaagggtc taagcgaaag 60
cgataagtat tccacctggg gagtacgtcg gcaacgatga aactcaaagg aattgacggg 120
ggcccgacac agcggaggaa catgtggttt aattcgatga tacgcgagga accttaccg 180
ggattgaaat ttagcgggag ggctatgaga gtacgctttc ctacgggact gctaagtagg 240
tgctgcatgg ttgtcgtcag ctctgtccgt gaggtgttgg ctttaagtgc ataacgagcg 300
caaccacagt tgatagttac taacagttaa agctgaggac tctatcgaga cagccggcgt 360
aagccgtgag gaaggtgtgg atgacgtcaa atcagcacgg cccttacatc cggggcgaca 420
cacgtgttac aatggtgagg acagcgggaa gcggcctggg gacaggtagc agatcccaaa 480
acctcatccc agttcggatt ggagtctgca actcgactct atgaagctgg attcgctagt 540
aatcgcgcat cagccatggg gcggtgaata c 571

```

<210> 95

<211> 1024

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: *P. gulae* B43  
fimA polynucleotide sequence

<400> 95

```

tctaaatcga aaaagatcct aataaaacaa tattcacttt taaaacaaaa acgagatgaa 60
aaagactaag tttttcttgt tgggacttgc tgcccttgct atgacagctt gtaacaaaga 120
caacgaagca gaaccggttg tagaaggtaa cgctaccatt agcgtagtat tgaagaccag 180
caatccgaat cgtgctttcg gggttgcaga tgacgaagca aaagtggcta aactgactgt 240
aatgggtctac aagggtgagc agcaggaagc catcaaata gccgaaaatg caattaaggt 300
tgagaacatc aaatgtgggtg caggctcacg tacgctgggc gtaatggcca atacgggtgg 360

```



```

aatggaattg gctggcaaga ctcttgcaga ggtaaaagca ttgacaactg aactaactgc 420
agaaaaccaa gaggtacag gtttgatcat gacagcagag cctgttgacg taacacttgt 480
cgccggcaat aactattatg gttatgatgg aactcaggga ggcaatcaga tttcgcaagg 540
tactcctctt gaaatcaaac gtgttcatgc ccgtattgcg ttcaccaaga ttgaagtga 600
gatgagcgag tcttatgtga acaaatacaa ctttaccccc gaaaacatct atgcacttgt 660
ggctaagaag aagtctaato tattcggtac ttcattggca aatagtgatg atgcttattt 720
gaccggttct ttgacgactt tcaacggtgc ttatacccct gcaaactata ctcatgtcgt 780
ctggttgga agaggctaca cagcgccttc caatgatgct ccacaagggt tctatgtttt 840
ggagagtgca tacgctcaga atgcaggctc acgtcctacc attctatgtg taaagggtaa 900
gctgacaaag catgatggta ctcttttgag ttctgaggaa atgacagctg cattcaatgc 960
cggctggatt gttgcaaaca atgatcctac gacctattat cctgtattag tgaactttga 1020
gagc 1024

```

<210> 96

<211> 733

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P.

circumdentaria B52 fimA polynucleotide sequence

<400> 96

```

taatggagaa cagcaggaag ccatcgaatc agccgaaaat gcgactaaga ttgagaatat 60
caaatgtggg gcaggccaac gtacgctggg cgtaatggcc aatacgggtg gaatggaatt 120
ggctggcaag actcttgcag aggtaaaagc attgacaact gtactgactg aagaaaacca 180
agaggccaca ggtttgatca tgacagcaga gccaaaagca atcgttttga aggcaggcaa 240
gaactatatt ggatacgatg gagccggaga gggcaaccac attgagaatg ctctcttga 300
aatcaaactg gtacatgctc gcatggcttt caccgaaatt aaagtacaga tgagcgcagc 360
ctacgataac atttacacat ttaccctga aaagatttat ggtctcattg caaagaagca 420
atctaatttg ttcggggcaa cactcgtgaa tgcagacgct aattatctga caggttcttt 480
gaccacattt aacggtgctt acacacctac caactatgcc aatgttcctt gggtgagccg 540
tgattacgtt gcacctaccg ctggtgctcc tcagggttc tacgtattag aaaatgacta 600
ctcagctaac agtggacta ttcattccgac aatcctgtgt gtttatggca aacttcagaa 660
aaacggagcc gacctgacgg gaaccgattt agcagcagct caggccgcca attgggtgga 720
tgagaaggc aag 733

```

<210> 97

<211> 1024

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. guiae B69

fimA polynucleotide sequence

&lt;400&gt; 97

```

ggcgcagcat aacctcgacg aactgcgaca ctatatgcag gacaatctct aaatcgaata 60
aagattctaa taaaacaata ttcactttta aaacaaaaac aagatgaaaa agactaagtt 120
tttcttggtg ggacttgctg cccttgctat gacagcttgt aacaaagaca acgaagcaga 180
acccgttgta gaaggtaacg ctaccattag cgtagtattg aagaccagca atccgaatcg 240
tggttttcggg gttgcagatg acgaagcaaa agtgggctaag ttgaccgtaa tggttttataa 300
tggagaacag caggaagcca tcgaatcagc cgaaaatgcg actaagattg agaatatcaa 360
atgtggtgca ggccaacgta cgctggctcg aatggccaat acgggtggaa tgggaattggc 420
tggcaagact cttgcagagg taaaagcatt gacaactgta ctgactgaag aaaaccaagg 480
ggccacaggt ttgatcatga cagcagagcc aaaagcaatc gttttgaagg caggcaagaa 540
ctatatggga tacgatggag cgggagaggg caaccacatt gagaatgctc ctcttgaaat 600
caaacgtgta catgctcgca tggctttcac cgaaattaaa gtacagatga gcgcagccta 660
cgataacatt tacacattta cccctgaaaa gatttatggg ctcatgcaa agaagcaatc 720
taatttggtt ggggcaacac tcgtgaatgc agacgctaata tatctgacag gttctttgac 780
cacatttaac ggtgcttaca cacctaccaa ctatgccaat gttccttggt tgagccgtga 840
ttacgttgca cctaccgctg gtgctcctca gggcttctac gtattagaaa atgactactc 900
agctaacagt ggaactattc atccgacaat cctgtgtgtt tatggcaaac ttcagaaaaa 960
cggagccgac ctgacgggaa ccgatttagc agcagctcag gccgccaatt ggggtggatgc 1020
agaa 1024

```

&lt;210&gt; 98

&lt;211&gt; 733

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:P.

circumdentaria B97 fimA polynucleotide sequence

&lt;400&gt; 98

```

taatggagaa cagcaggaag ccacgaatc agccgaaaat gcgactaaga ttgagaatat 60
caaatgtggt gcaggccaac gtacgctggt cgtaatggcc aatacgggtg gaatggaatt 120
ggctggcaag actcttgacg aggtaaaagc attgacaact gtactgactg aagaaaacca 180
agaggccaca ggtttgatca tgacagcaga gccaaaagca atcgttttga aggcaggcaa 240
gaactatatt ggatacgatg gagccggaga gggcaaccac attgagaatg ctctcttga 300
aatcaaagct gtacatgctc gcatggcttt caccgaaatt aaagtacaga tgagcgcagc 360
ctacgataac atttacacat ttacccttga aaagatttat ggtctcattg caaagaagca 420
atctaatttg ttcggggcaa cactcgtgaa tgcagacgct aattatctga caggttcttt 480
gaccacattt aacggtgctt acacacctac caactatgcc aatgttcctt ggttgagccg 540
tgattacgtt gcacctaccg ctggtgctcc tcagggttcc tacgtattag aaaatgacta 600
ctcagctaac agtggaaacta ttcacccgac aatcctgtgt gtttatggca aacttcagaa 660
aaacggagcc gacctgacgg gaaccgattt agcagcagct caggccgcca attgggtgga 720
tgcagaaggc aag 733

```

&lt;210&gt; 99

&lt;211&gt; 1024

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P.  
cangingivalis B98 fimA polynucleotide sequence

&lt;400&gt; 99

```

ggcctcgaga acaaagacaa cgaagcagaa cccgttgtag aaggtaacgc taccattagc 60
gtagtattga agaccagcaa tccgaatcgt gctttcgggg ttgcagatga cgaagcaaaa 120
gtggctaaac tgactgtaat ggtctacaag ggtgagcagc aggaagccat caaatcagcc 180
gaaaaatgcaa ttaagggttg gaacatcaaa tgtggtgcag gctcacgtac gctggtcgta 240
atggccaata cgggtggaat ggaattggct ggcaagactc ttgcagaggt aaaagcattg 300
acaactgaac taactgcaga aaaccaagag gctacagggt tgatcatgac agcagagcct 360
gttgacgtaa cacttgctgc cggcaataac tattatgggt atgatggaac tcagggaggc 420
aatcagattt cgcaaggtag tcctcttgaa atcaaactg ttcatgcccg tattgcgttc 480
accaagattg aagtgaagat gagcagagtct tatgtgaaca aatacaactt taccctcgaa 540
aacatctatg cacttggtgg taagaagaag tctaacttat tcggtacttc attggcaaat 600
agtgatgatg cttatttgac cggttctttg acgactttca acggtgctta tacccttgca 660
aactatactc atgtcgtctg gttgggaaga ggctacacag cgccttccaa tgatgctcca 720
caaggtttct atgttttgga gagtgcatac gctcagaatg caggtctacg tcctaccatt 780
ctatgtgtaa agggtaagct gacaaagcat gatggtactc ctttgagtgc tgaggaaatg 840
acagctgcat tcaatgccgg ctggattggt gcaaacaatg atcctacgac ctattatcct 900
gtattagtga actttgagag caataattac acctacacag gtgatgctgt tgagaaaggg 960
aaaatcggtc gtaaccacaa gtttgacatc aatctgacga tcaccgggtc tggtagaat 1020
aatc 1024

```

&lt;210&gt; 100

&lt;211&gt; 783

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. salivosa  
B104 fimA polynucleotide sequence

&lt;400&gt; 100

```

tggctaartt gactgtaatg gtttataatg gagaacagca ggaagccatc raatcagccg 60
aaaatgagac taagrttgar rayatcaaat gtrgtgcagg ccaacgtacg ctggtcgtaa 120
tggccaatac ggggtgsaatg gaaytggytg gcaagactct tgcagaggta aaagcattga 180
caactgwact gactgmagaa aaccaagagg cyrcaggktt gatcatgaca gcagagccaa 240
aarcaatcgt tttgaaggca ggcaagaact ayattggata crrtggarcc ggagagggga 300
aycacattga gaatgmtcct cttraratca arcgtgtwca tgctcgcatt gctttcaccg 360
aaattaaagt rcaratgagc gcagcctacg ataacattta cacattyryc cctgaaaaga 420
tttatgggtc cattgcaaag aagcaatcta atttgttcgg ggcaaacactc gtraatgcag 480
acgctaatta tctgacaggt tctttgacca catttaacgg tgcttacaca cctrecaact 540
atgccaatgt kccctggytg agccgtratt acgttgacac tccgcygrt gctcctcagg 600

```

```

gyttctacgt attagaaaat gactactcag ctaacrgtgg aactattcat cgcacaatcc 660
tgtgtgttta tggcaaaactt cagaaaaacg gagccgacyt ggcgggarcc gatttagcar 720
cwgctcaggc cgccaattgg gtggatgcag aaggcaagac ctattaccct gtattgrtra 780
act 783

```

&lt;210&gt; 101

&lt;211&gt; 733

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. denticanis  
B106 fimA polynucleotide sequence

&lt;400&gt; 101

```

taatggagaa cagcaggaag ccatcgaatc agccgaaaat gcgactaaga ttgagaatat 60
caaatgtggt gcaggccaac gtacgctggt cgtaatggcc aatacgggtg gaatggaatt 120
ggctggcaag actcttgacg aggtaaaagc attgacaact gtactgactg aagaaaacca 180
agaggccaca ggtttgatca tgacagcaga gccaaaagca atcgttttga aggcaggcaa 240
gaactatatt ggatacgatg gagccggaga gggcaaccac attgagaatg ctctcttgga 300
aatcaaactg gtacatgctc gcatggcttt caccgaaatt aaagtacaga tgagcgcagc 360
ctacgataac atttacacat ttacccttga aaagatttat ggtctcattg caaagaagca 420
atctaatttg ttccggggcaa cactcgtgaa tgcagacgct aattatctga caggttcttt 480
gaccacattt aaögggtgctt acacacctac caactatgcc aatgttcctt ggttgagccg 540
tgattacggt gcacctaccg ctgggtgctcc tcagggcttc tacgtattag aaaatgacta 600
ctcagctaac agtggaacta ttcacccgac aatcctgtgt gtttatggca aacttcagaa 660
aaacggagcc gacctgacgg gaaccgattt agcagcagct caggccgcca attgggtgga 720
tgcagaaggc aag 733

```

&lt;210&gt; 102

&lt;211&gt; 742

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. endodontalis  
B114 fimA polynucleotide sequence

&lt;400&gt; 102

```

caagggtgag cagcaggaag ccatcaaatc agccgaaaat gcaattaagg ttgagaacat 60
caaatgtggt gcaggctcac gtacgctggt cgtaatggcc aatacgggtg gaatggaatt 120
ggctggcaag actcttgacg aggtaaaagc attgacaact gaactaactg cagaaaacca 180
agaggctaca ggtttgatca tgacagcaga gcctgttgac gtaacacttg tcgccggcaa 240
taactattat ggttatgatg gaactcaggg aggcaatcag atttcgcaag gtactcctct 300
tgaaatcaaa cgtgttcattg cccgtattgc gttcaccaag attgaagtga agatgagcga 360
gccttatgtg aacaaatata actttacccc cgaaaacatc tatgcacttg tggctaagaa 420

```

```

gaagtctaatt ctattcggtta cttcattggc aaatagtgat gatgcttatt tgaccgggttc 480
tttgacgact ttcaacgggtg cttatacccc tgcaaactat actcatgtcg tctgggttggg 540
aagaggctac acagcgcctt ccaatgatgc tccacaaggt ttctatgttt tggagagtg 600
atacgtcag aatgcaggtc tacgtcctac cattctatgt gtaaagggtta agctgacaaa 660
gcatgatggg actcctttga gttctgagga aatgacagct gcattcaatg ccggctggat 720
tgttgcaaac aatgataccta cg 742

```

<210> 103

<211> 281

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. gulae B43  
FimA polypeptide sequence

<400> 103

```

mkktkgaaam tacnkdnvav gnatsvvkts nnragvadda kvaktvmvyk gaksanakvn 60
kcgagsrtvv mantggmagk tavkatttan atgmtavdvt vagnnygyd gtggnsgrkr 120
vharatkvmk ssyvnkyntn yavakksng tsansddayt gsttngayta nythvwwgrg 180
ytasndagyv sayanagrtc vkgktkhdgt ssmtaanagw vanndttyyv vnsnnytytg 240
davkgkvrnh kdnttggttn ntsannvncv vaawkgvvnv w 281

```

<210> 104

<211> 170

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P.  
circumdentaria B52 FimA polypeptide sequence

<400> 104

```

ngasanatkn kcgagrtvmm antggmagkt avkattvtna tgmtakavka gknygydgag 60
gnhnakrvha rmatkvmsaa ydnyttkyga kksngatvna danytgsttn gayttnyanv 120
wsrdyvtag agyvndysan sgthtcvyvk kngadtgtta aaaaanwvdag 170

```

<210> 105

<211> 275

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. gulae B69  
FimA AA

&lt;400&gt; 105

mkkktkgaaam tacnkdnvavv gnatsvvkts nnrvgvadda kvaktvmvyn gasanatknk 60  
 cgagrtvma ntggmagkta vkattvtnga tgmtakavka gknygydgag gnhnakrvha 120  
 rmatkvmsaa ydnyttkyga kksngatvna danytgsttn gayttnyanv wsrdivatag 180  
 agyvndysan sgthtcvygk kngadtgtada aaaanwvdag ktyyvvnnsn nytydngytk 240  
 nkrnhkydkt tggtnnntsa hnvtvawvv gnatw 275

&lt;210&gt; 106

&lt;211&gt; 170

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P.  
 circumdentaria B97 FimA polypeptide sequence

&lt;400&gt; 106

ngasanatkn kcgagrtvmm antggmagkt avkattvtna tgmtakavka gknygydgag 60  
 gnhnakrvha rmatkvmsaa ydnyttkyga kksngatvna danytgsttn gayttnyanv 120  
 wsrdivatag agyvndysan sgthtcvygk kngadtgtada aaaanwvdag 170

&lt;210&gt; 107

&lt;211&gt; 257

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P.  
 cangingivalis B98 FimA AA

&lt;400&gt; 107

vvgnatsvvk tsnnragvad dakvaktvmv ykgaksanak vnkcagsrt vvmantggma 60  
 gktavkattdt anatgmtavd vtvagnnyyg ydgtggnsgr krvharatk kmssyvnkyn 120  
 tnyavakkks ngtsansdda ytgsttngay tanythvwwg rgytasndag yvsayanagr 180  
 tcvkgkthkd gtssmtaana gwvanndtty yvvnnsnyty tgdavkgkvr nhkdnttgg 240  
 nnntsannvn cvvaawk 257

&lt;210&gt; 108

&lt;211&gt; 161

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. salivosa

## B104 FimA polypeptide sequence

&lt;400&gt; 108

atvmvyngas anatkkcagr tvvmantgmg ktavkatttn agmtakvkag kngyggghnr 60  
 vharatkvm saaydnytky gaksngatv nadanytgst tngaytnyan vwsryvaaag 120  
 yvndysangt htcvygkng adgdaaaanw vdagktyyv n 161

&lt;210&gt; 109

&lt;211&gt; 170

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. denticanis  
 B106 FimA polypeptide sequence

&lt;400&gt; 109

ngasanatkn kcgagrtvrm antggmagkt avkattvtna tgmtakavka gknygydgag 60  
 gnhnakrvha rmatkvmsaa ydnyttkyga kksngatvna danytgsttn gayttngayv 120  
 wsrdivatag agyvndysan sgthtcvygk kngadtgtda aaaanwvdag 170

&lt;210&gt; 110

&lt;211&gt; 177

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. endodontalis  
 B114 FimA polypeptide sequence

&lt;400&gt; 110

kgaksanakv nkcagagrtv vmantggmag ktavkatttna natgmtavdv tvagnnyygy 60  
 dgtggngsgtk rvharatkvk mssyvnynt nyavakksn gtsansdday tgsttngayt 120  
 anythvvwgr gytasndagy vsayanagrt cvkgktkhdg tssmtaanag wvanndt 177

&lt;210&gt; 111

&lt;211&gt; 1024

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. gulae B43  
 oprF polynucleotide sequence

&lt;400&gt; 111

```

acattcgttg gagctattgc actgaatgca agtgcacagg aaaataactgt accggcaacg 60
ggtcagttac ccgcaaaaaa tgttgctttc gtcgcaaca aagcaggcag caattgggtc 120
gtaacactgc agggcggtgt tgcagcgcag ttctcfaatg acaacaacaa caaagatttt 180
gtagaccgct tgggtgctgc cggctctatt tcagttggaa aatatcaciaa tccattcttt 240
gcaaccctgt tgcaaattaa cggagctcag gcacacacgt tccttggaaa aaatgcggaa 300
caagaaatta agaccaattt tggcgcagct cactttgact tcatgttcga tgtggttaat 360
tactttgcgc catatcgaga aaatcgtttc ttccatttaa ttccatgggt aggtgttggt 420
taccagcata aattcattgg cagcaaatgg agtaaagaca atgtcgagtc tctgactgcc 480
aatctgggtg ttatgatggc ttccagatta ggaaaacgtg tagactttgt gatcgaagca 540
caagcagcac actccaatct caacttaagc cgtgctttca atgccaagcc gactcctatt 600
ttccaggatc aggaaggacg ttattacaat ggattccaag gaatggcgac agcagggtctt 660
aacttccgct tgggtgctgt aggttcaat gccatcgagc ccatggacta cgcgcttatt 720
aacgatctga atggtcagat taatcgctg cgcagagaag tcgaagaact ctccaagcgt 780
cctgtatcat gtcccgaatg ccccgacgtt acaccggtta ccaagacaga aaacaagcta 840
accgagaagg ctgtactctt ccgtttcgac agctatgttg tagacaaaga ccagcttatt 900
aatctgtatg acgtagctca gtttgtaaaa gaaaccaacg agccgattac tgttgtaggc 960
tatgctgatc ctacgggtga cactcagtag aacgaaagat tgtctgagcg tcgcgcaaaa 1020
gccg 1024

```

<210> 112

<211> 1024

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. cansulci B46  
oprF polynucleotide sequence

<400> 112

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acattggccg gggtttacgc cctttcagcc tctgctcagc aggagaatat gccacgaatg 60
gggcagactc ccgcaagaa taccgcttac gtcgctctg aagccggtga caattgggtt 120
gtgactttgc aaggaggtgc tgctatgcag tttgggaaag gtaacgagga tgcgacttc 180
ttcgaccgcc aaactgttgc tcccactttt gccgtaggta aatggcaciaa tcctttcttc 240
gggaccagat tgcaaattggg cttgggggta tctcacgact tctcgaaciaa cgaagcgaaa 300
tccaagtggg agatgaacca cgctcgctat gctaacgcac actttgactt tatgtttgat 360
gtgattaact acttcaagcc ctacagttag gaccgcgtat tccaccttat tccgtgggta 420
ggtttggtt acgatcacia gtttgagaaa aacagcaact tcaaggtgga tgctcttaca 480
gccaacgccg gtttgatgtt tgctttccgt gtgatggagc gtatggacat tgtgttgga 540
agccaggtaa tgtattctga cttcaacctc aacacagctc tgcccgagcc tcgctacaca 600
gcttgctccg gcatgctcac tgccggtttg aacttccgta taggaaatat cggatggagc 660
gagatcctac caatggattg gggcttggtg aatgacctga acggacaaat caacgccatg 720
cgtgctaaga acgcagagtt gagcaagcgt cccgtttctt gcccgaatg cccggaagtt 780
gagcctcgtg tagagcgtat caatatgctt tcggacaagt ctgttctttt ccgtgccggc 840
aagacaactg tagacagcga tcaaattggtg acgatcttcg acgtagctca gtttgcaaag 900
aagaatggca cacagatcac cgttacaggc tatgcagaca agaagggcaa agaaagcgat 960
cgcacctctg aacttcgtgc aaaagccgta gccaaagattc tcaccgacia gtacggtgta 1020
cctt 1024

```



<210> 113  
 <211> 1024  
 <212> DNA  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P.  
 circumdentaria B52 oprF polynucleotide sequence

<400> 113  
 tctataatgg gagctacagc actctccgcg agtgcctcaac aatctacgac acctgagact 60  
 caaactttgc cagctcgcaa gacggctttt gaccgttccg cgggtcactg gttcttgact 120  
 ctacaggggtg gtgtaaatgc acagtttttg gaagaaaacg agtctcaaga catcgtaaata 180  
 cgtctccgtg tgatgccaac tctttcttta ggaaagtggc acaatcccta ttttgcaacc 240  
 cgtttgcaag tttttggggg gccaaacccct acttactaca aggaggtttc tggggaggtt 300  
 aagaccctaa ataccgccat ggctggagct cactttgatt ttatgtttga tgtagtaaac 360  
 ttctatgcaa agtataatcc taaacgagta ttccatttga ttccttggtt cgggtgtggga 420  
 tatggtttca aatactataa cgattttgct gatttagctg atatgattca gtttaatgaa 480  
 cccttccgtc actcagcaac tgcgaatgct ggtttgatga tgagttttcg cttggcaaaa 540  
 cgtttgatt tggttctgga agggcaggct atatattcta acttgaatat tgtaaagcaa 600  
 gagatagatt ataaagcccc cattatgccc tattcaaata tctacaacgg attgacaggt 660  
 gtcgttactg caggtctcaa ctttaatctc ggtcgtgttg cttgggagtc cgtaactcct 720  
 atggatatgg atcttattaa tgacctaaac ggacaaatta accgtttgcg ttctgagaat 780  
 acagagttga gaaaacgtcc agtttcttgc ccagaatgtc ctgaagttac tgcagagacg 840  
 gaagtagtta ctgaaaacgt tttaggtgat aaggcgattg ttttcaagtt taatagcgca 900  
 actattgaca aagatcaaca cattgttttg caggatatcg ctgactttgt taaagatggc 960  
 aacaaagcta ttgttgtaat aggcttcgca gatacaacag gtgatattaa ttacaatatg 1020  
 catt 1024

<210> 114  
 <211> 1024  
 <212> DNA  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. guiae B69  
 oprF polynucleotide sequence

<400> 114  
 acattcggtg gagctattgc actgaatgca agtgcacagg aaaatactgt accggcaacg 60  
 ggtcagttac ccgcaaaaaa tggtgctttt gcccgcaata aagcaggcgg caattgggtt 120  
 gtaacactgc aaggtgggtg tgcagcacag ttccttaatg acaacaacaa caaagatcta 180  
 gtagaccgct taggagctac cggatctatc tccgttgga aatatcaciaa tccattcttt 240  
 gogactcgtt tgcaaatataa cggaggtcaa gcacacacgt tccctgggaa gaatgcggaa 300  
 caagaaatta acaccaatth tggagcagct cactttgact tcatgttcga tgtgggtaac 360

```

tactttgcg ccatatcgga aaaccgtttc ttccatttaa ttccatgggt aggtgttggt 420
taccaacaca aattcatcgg tagcgaatgg agtaaagaca acgtcgagtc gctgaccgca 480
aacatgggtg ttatgatggc ttccagatta ggggaagcgcg tggactttgt gatcgaagca 540
caagctgctc actccaatct taattttaagt cgcgcattca atgccaagaa aactcctatt 600
ttccacgata aagaaggctc ctattacaat ggattccaag gaatggctac agcgggtctt 660
aacttccgct taggtgctgt tggcttcaat gccatcgagc caatggacta cgcgcttata 720
aacgatctga atggtcagat taaccgtttg cgcagagaag ttgaagagct ctctaagcgt 780
cctgtatcat gccccgaatg tcccgatgta acaccggtta ctaagacaga aaacaagcta 840
accgagaagg ctgtactctt ccgcttcgac agctatgttg tagacaaaga ccagctgata 900
aatctgtatg acgttgctca gttcgtaaaa gaaactaacg aaccgattac cgttgtaggt 960
tatgccgata ctacgggcag cactcagtag aacgaaagat tgtctgagcg tcgcgcaaaa 1020
gccg

```

<210> 115

<211> 1024

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P.

circumdentaria B97 oprF polynucleotide sequence

<400> 115

```

tctgttatgg gagctacagc actcacagtt agtgctcagc aacctactac acctgagact 60
cagacattgc ctgctcataa gacggctttt gaccgttctg caggacattg gttcttgact 120
ctccaagggtg gagttagtg ctaattttta gaagaaaatg aaagtcaaga aatcttgaat 180
cgtcttcatg ttatgcctac aatctcttta ggcaagtggc acaatcctta ttttgcaact 240
cgtttgcaag tgttcggagg tctactcct actttttata agaatgctgc tggtaagggtg 300
atgaaggaaa atgcggctat ggctggggct cactttgact ttatgtttga tgttgtaaac 360
tactttggta agtataatcc aaagagagtc ttcatcttg tgcttggtt cgggtgttga 420
tatggcttta aataccataa tgatttcgcc gaaatgagtg atatcattaa gtttaatgag 480
ccttatcgcc attcagcaac agcgaatgca gggttgatga tgagtttccg cttagcaaaa 540
cgtcttgatt tagtgcttga aggacaggct atatattcta atttgaatat tgttaagcaa 600
gaaattgatt ataaagctcc ttctactcct tattctccaa attataatgg gcttttggga 660
gttggttacag caggctctta ctttaatctt ggtcgtgttg cttgggagac tgttactccc 720
atggatatgg atttgattaa tgatcttaat ggtcaaatca atcgtttgcg ttctgagaat 780
actgagttga gaaaacgtcc tgtttcttgt cctgaatgcc cagaagtttc taaagaaaca 840
actgtagtta cagaaaatgt attgggagac aaagctattg ttttcaaatt taatagtgca 900
actatcagca aagatcaaca tattgttttg caagacattg cggactttgt taagaatgga 960
aataaggggg ttgccgtgat aggtttcgca gatgtaacag gagatgcca ttacaatatg 1020
caac

```

<210> 116

<211> 948

<212> DNA

<213> Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:P.

cangingivalis B98 oprF polynucleotide sequence

&lt;400&gt; 116

```

ggtggagtta gtgctcaatt tttagaagaa aatgaaagtc aagaaatctt gaatcgtctt 60
catgttatgc ctacaatctc tttaggcaag tggcacaatc cttattttgc aactcgtttg 120
caagtgttcg gaggtcctac tcctactttt tataagaatg ctgctggtaa ggtgatgaag 180
gaaaatgcgg ctatggctgg ggctcacttt gactttatgt ttgatgttgt gaactacttt 240
ggtaagtata atccaaagag agtctttcat cttgtgcctt gggtcgggtg tggatatggc 300
tttaaatacc ataatgattt cgccgaaatg agtgatatca ttaagtttaa tgagccttat 360
cgccattcag caacagcgaa tgcagggttg atgatgagtt tccgcttagc aaaacgtctt 420
gatttagtgc ttgaaggaca ggctatatat tctaatttga atattgttaa gcaagaaatt 480
gattataaag ctccctctac tccttattct ccaaattata atgggctttt gggagtgtgt 540
acagcaggtc ttaactttaa tcttggtcgt gttgcttggg agactgttac tcccatggat 600
atggatttga ttaatgatct taatggtaa atcaatcgtt tgcgttctga gaatactgag 660
ttgagaaaac gtctgtttc ttgtcctgaa tgcccagaag tttctaaaga aacaactgta 720
gttacagaaa atgtattggg agacaaagct attgttttca aatttaatag tgcaactatc 780
agcaaagatc aacatattgt tttgcaagac attgcggact ttgttaagaa tggaaataag 840
ggggttgccg tgatagggtt cgcatagata acaggagatg ccaattacaa tatgcaactt 900
tctgaacgtc gtgctaaggc tgttgcgga gctcttgtga atcaattc 948

```

&lt;210&gt; 117

&lt;211&gt; 969

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:P. salivosa

B104 oprF polynucleotide sequence

&lt;400&gt; 117

```

cattggttct tgactctcca aggtggagtt agtgctcaat ttttagaaga aaatgaaagt 60
caagaaatct tgaatcgtct tcatgttatg cctacaatct ctttaggcaa gtggcacaat 120
cctattttgc caactcgttt gcaagtgttc ggaggtccta ctccactttt ttataagaat 180
gctgctggta aggtgatgaa ggaaaatgcg gctatggctg gggctcactt tgactttatg 240
tttgatgttg tgaactactt tggttaagat aatccaaaga gagtctttca tcttgtgcct 300
tggttcgggtg ttggatatgg ctttaaatac cataatgatt tcgccgaaat gagtgatata 360
attaagttaa atgagcctta tcgccattca gcaacagcga atgcagggtt gatgatgagt 420
ttccgcttag caaaacgtct tgatttagtg cttgaaggac aggctatata ttctaatttg 480
aatattgtta agcaagaaat tgattataaa gctccttcta ctccattatc tccaaattat 540
aatgggcttt tgggagtgtg tacagcaggt cttaacttta atcttggctg tgttgccctg 600
gagactatta ctcccatgga tatggatttg attaatgata ttaatggta aatcaatcgt 660
ttgcgttctg agaatactga gttgagaaaa cgtcctgttt cttgtcctga atgccagaa 720
gtttctaaag aaacaactgt agttacagaa aatgtattgg gagacaaagc tattgttttc 780
aaatttaata gtgcaactat cagcaaagat caacatattg ttttgcaaga cattgcggac 840

```

```

tttgттаага atggaaataa gggggttgcc gtgatagggt tcgcagatgt aacaggagat 900
gccaaattaca atatgcaact ttctgaacgt cgtgctaagg ctgttgcgga agctcttgtg 960
aatcaattc 969

```

&lt;210&gt; 118

&lt;211&gt; 1024

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. denticanis  
B106 oprF polynucleotide sequence

&lt;400&gt; 118

```

gctcataaga cggcttttga cgtttctgca ggacattggt tcttgactct ccaaggtgga 60
gttagtgctc aattttttaga agaaaatgaa agtcaagaaa tcttgaatcg tcttcatgtt 120
atgcctacaa tctcttttagg caagtggcac aatccttatt ttgcaactcg tttgcaagtg 180
ttcggagggtc ctactcctac tttttataag aatgctgctg gtaaggtgat gaaggaaaat 240
gcggctatgg ctgggggtca ctttgacttt atgtttgatg ttgtgaacta ctttggttaag 300
tataatccaa agagagtctt tcatcttgtg ccttggttcg gtgttgata tggttttaa 360
taccataatg atttcgccga aatgagtgat atcattaagt ttaatgagcc ttatcgccat 420
tcagcaacag cgaatgcagg gttgatgatg agtttccgct tagcaaacg tcttgattta 480
gtgcttgaag gacaggctat atattctaatt ttgaatattg ttaagcaaga aattgattat 540
aaagctcctt ctactcctta ttctccaaat tataatgggc ttttgggagt tggtacagca 600
ggtcttaact ttaatcttgg tcgtgttgct tgggagactg ttactcccat ggatatggat 660
ttgattaatg atcttaatgg tcaaatcaat cgtttgcgtt ctgagaatac tgagttgaga 720
aaacgtcctg tttcttgtcc tgaatgccca gaagtttcta aagaaacaac tgtagttaca 780
gaaaatgtat tgggagacaa agctattgtt ttcaaattta atagtgcac tatcagcaaa 840
gatcaacata ttgttttgca agacattgct gactttgtta agaatggaaa taagggggtt 900
gccgtgatag gtttcgcaga tgtaacagga gatgccatt acaatatgca actttctgaa 960
cgtcgtgcta aggtgttgc ggaagctctt gtgaatcaat tcggagttcc ttctgatatg 1020
attt 1024

```

&lt;210&gt; 119

&lt;211&gt; 1024

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. endodontalis  
B114 oprF polynucleotide sequence

&lt;400&gt; 119

```

tcagcactgg gggcttttggc acttacagct agtgctcaac aaactacgaa accagcgaat 60
agtatgcccg cattcaagac tgcatttgaa cgcagcggcg gtcattgggt tctgacaatt 120
caggggtggcc tgagtgtcga acttttgggt gaaaatgaaa agatggactt tggcaagcgt 180

```

```

ctgctacatg ctgccaaaggc cagtgcacaac acccaaacag aggctagcta cctacgcac 240
atgcccacgc tctctgtagg taaatggcat aatccctact ttgctactcg tgtacagctc 300
ttcggtggtc tcaactcctct ctacaatact gaggggtggcg ttaatgtaca cacctacaac 360
actgccacga tcggtgcccc ctatgatttc atgtttgatg tagtaaaacta tttcgccaag 420
tacaaccccc aacgttttctt ccacgtaatt ccttgggtgg gtcttggtta caacttcaag 480
tatcatgatg tatttggatt caaggagccc tatcgtcact ctgtcacagg taacgcaggc 540
atggagtttg ctttccgcct cggtaagcgt gtagaccttg tactcgaagc tcaggtagtg 600
tacaacaacc tgaacctgat caagcaggaa gtcgactacg atgtagtcac tactccctat 660
gtacctgctg atacatacgc tggctcttatg accatgttta ctgctggtct taacttcaat 720
ctgggcaagg ttgagtggga aactgttgag ccgatggact accagctcat aaacgacttg 780
aactctcaga tcagccgtct acgtagcgaa aacgcagagc tttccaagcg tccgtctttc 840
tgccccgagt gtcccgaagt agaggaagta gaagatgttg ttgttgacca gtatgtcctc 900
accgacaagg ctatcctctt cgactttgac aagagcaaca tccgcaagga ccaacaagct 960
cagcttggtg tgattgctga attcgtgaag aagtacaata cgcctatcgt ggtagtaggc 1020
tatg 1024

```

&lt;210&gt; 120

&lt;211&gt; 375

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:P. guiae B43

OprF polypeptide sequence

&lt;400&gt; 120

```

Thr Phe Val Gly Ala Ile Ala Leu Asn Ala Ser Ala Gln Glu Asn Thr
  1             5             10             15

```

```

Val Pro Ala Thr Gly Gln Leu Pro Ala Lys Asn Val Ala Phe Ala Arg
          20             25             30

```

```

Asn Lys Ala Gly Ser Asn Trp Phe Val Thr Leu Gln Gly Gly Val Ala
          35             40             45

```

```

Ala Gln Phe Leu Asn Asp Asn Asn Asn Lys Asp Phe Val Asp Arg Leu
          50             55             60

```

```

Gly Ala Ala Gly Ser Ile Ser Val Gly Lys Tyr His Asn Pro Phe Phe
          65             70             75             80

```

```

Ala Thr Arg Leu Gln Ile Asn Gly Ala Gln Ala His Thr Phe Leu Gly
          85             90             95

```

```

Lys Asn Ala Glu Gln Glu Ile Lys Thr Asn Phe Gly Ala Ala His Phe
          100            105            110

```

Asp	Phe	Met	Phe	Asp	Val	Val	Asn	Tyr	Phe	Ala	Pro	Tyr	Arg	Glu	Asn	
	115						120					125				
Arg	Phe	Phe	His	Leu	Ile	Pro	Trp	Val	Gly	Val	Gly	Tyr	Gln	His	Lys	
	130					135					140					
Phe	Ile	Gly	Ser	Lys	Trp	Ser	Lys	Asp	Asn	Val	Glu	Ser	Leu	Thr	Ala	
145				150						155					160	
Asn	Leu	Gly	Val	Met	Met	Ala	Phe	Arg	Leu	Gly	Lys	Arg	Val	Asp	Phe	
			165						170					175		
Val	Ile	Glu	Ala	Gln	Ala	Ala	His	Ser	Asn	Leu	Asn	Leu	Ser	Arg	Ala	
		180						185					190			
Phe	Asn	Ala	Lys	Pro	Thr	Pro	Ile	Phe	Gln	Asp	Gln	Glu	Gly	Arg	Tyr	
	195						200					205				
Tyr	Asn	Gly	Phe	Gln	Gly	Met	Ala	Thr	Ala	Gly	Leu	Asn	Phe	Arg	Leu	
	210					215					220					
Gly	Ala	Val	Gly	Phe	Asn	Ala	Ile	Glu	Pro	Met	Asp	Tyr	Ala	Leu	Ile	
225					230					235					240	
Asn	Asp	Leu	Asn	Gly	Gln	Ile	Asn	Arg	Leu	Arg	Arg	Glu	Val	Glu	Glu	
			245						250					255		
Leu	Ser	Lys	Arg	Pro	Val	Ser	Cys	Pro	Glu	Cys	Pro	Asp	Val	Thr	Pro	
			260					265					270			
Val	Thr	Lys	Thr	Glu	Asn	Lys	Leu	Thr	Glu	Lys	Ala	Val	Leu	Phe	Arg	
	275						280					285				
Phe	Asp	Ser	Tyr	Val	Val	Asp	Lys	Asp	Gln	Leu	Ile	Asn	Leu	Tyr	Asp	
	290					295					300					
Val	Ala	Gln	Phe	Val	Lys	Glu	Thr	Asn	Glu	Pro	Ile	Thr	Val	Val	Gly	
305					310					315					320	
Tyr	Ala	Asp	Pro	Thr	Gly	Asp	Thr	Gln	Tyr	Asn	Glu	Arg	Leu	Ser	Glu	
			325						330					335		
Arg	Arg	Ala	Lys	Ala	Val	Val	Asp	Val	Leu	Thr	Gly	Lys	Tyr	Gly	Val	
			340					345					350			
Pro	Ser	Glu	Leu	Ile	Ser	Val	Glu	Trp	Lys	Gly	Asp	Thr	Thr	Gln	Pro	
	355						360					365				

Phe Asn Lys Lys Ala Trp Asn  
370 375

<210> 121

<211> 366

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. cansulci B46  
OprF polypeptide sequence

<400> 121

Thr Leu Ala Gly Val Tyr Ala Leu Ser Ala Ser Ala Gln Gln Glu Asn  
1 5 10 15

Met Pro Arg Met Gly Gln Thr Pro Ala Lys Asn Thr Ala Tyr Ala Arg  
20 25 30

Ser Glu Ala Gly Asp Asn Trp Phe Val Thr Leu Gln Gly Gly Ala Ala  
35 40 45

Met Gln Phe Gly Lys Gly Asn Glu Asp Ala Asp Phe Phe Asp Arg Gln  
50 55 60

Thr Val Ala Pro Thr Phe Ala Val Gly Lys Trp His Asn Pro Phe Phe  
65 70 75 80

Gly Thr Arg Leu Gln Met Gly Leu Gly Val Ser His Asp Phe Ser Asn  
85 90 95

Asn Glu Ala Lys Ser Lys Leu Glu Met Asn His Ala Arg Tyr Ala Asn  
100 105 110

Ala His Phe Asp Phe Met Phe Asp Val Ile Asn Tyr Phe Lys Pro Tyr  
115 120 125

Ser Glu Asp Arg Val Phe His Leu Ile Pro Trp Val Gly Leu Gly Tyr  
130 135 140

Asp His Lys Phe Glu Lys Asn Ser Asn Phe Lys Val Asp Ala Leu Thr  
145 150 155 160

Ala Asn Ala Gly Leu Met Phe Ala Phe Arg Val Met Glu Arg Met Asp  
165 170 175

Ile Val Leu Glu Ser Gln Val Met Tyr Ser Asp Phe Asn Leu Asn Thr

180							185							190						
Ala	Leu	Pro	Glu	Pro	Arg	Tyr	Thr	Ala	Cys	Ser	Gly	Met	Leu	Thr	Ala					
195							200							205						
Gly	Leu	Asn	Phe	Arg	Ile	Gly	Asn	Ile	Gly	Trp	Ser	Glu	Ile	Leu	Pro					
210							215							220						
Met	Asp	Trp	Gly	Leu	Val	Asn	Asp	Leu	Asn	Gly	Gln	Ile	Asn	Ala	Met					
225							230							235						
Arg	Ala	Lys	Asn	Ala	Glu	Leu	Ser	Lys	Arg	Pro	Val	Ser	Cys	Pro	Glu					
245							250							255						
Cys	Pro	Glu	Val	Glu	Pro	Arg	Val	Glu	Arg	Ile	Asn	Met	Leu	Ser	Asp					
260							265							270						
Lys	Ser	Val	Leu	Phe	Arg	Ala	Gly	Lys	Thr	Thr	Val	Asp	Ser	Asp	Gln					
275							280							285						
Met	Val	Thr	Ile	Phe	Asp	Val	Ala	Gln	Phe	Ala	Lys	Lys	Asn	Gly	Thr					
290							295							300						
Gln	Ile	Thr	Val	Thr	Gly	Tyr	Ala	Asp	Lys	Lys	Gly	Lys	Glu	Ser	Asp					
305							310							315						
Arg	Thr	Ser	Glu	Leu	Arg	Ala	Lys	Ala	Val	Ala	Lys	Ile	Leu	Thr	Asp					
325							330							335						
Lys	Tyr	Gly	Val	Pro	Ser	Asp	Arg	Ile	Ser	Ile	Glu	Trp	Lys	Gly	Val					
340							345							350						
Ser	Glu	Gln	Val	Tyr	Asp	Asn	Arg	Asp	Trp	Asn	Arg	Val	Val							
355							360							365						

<210> 122

<211> 382

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P.

circumdentaria B52 OprF polypeptide sequence

 $\langle 400 \rangle$  122

Ser Ile Met Gly Ala Thr Ala Leu Ser Ala Ser Ala Gln Gln Ser Thr  
1 5 10 15



Thr Pro Glu Thr Gln Thr Leu Pro Ala Arg Lys Thr Ala Phe Asp Arg  
 20 25 30  
 Ser Ala Gly His Trp Phe Leu Thr Leu Gln Gly Gly Val Asn Ala Gln  
 35 40 45  
 Phe Leu Glu Glu Asn Glu Ser Gln Asp Ile Val Asn Arg Leu Arg Val  
 50 55 60  
 Met Pro Thr Leu Ser Leu Gly Lys Trp His Asn Pro Tyr Phe Ala Thr  
 65 70 75 80  
 Arg Leu Gln Val Phe Gly Gly Pro Thr Pro Thr Tyr Tyr Lys Glu Val  
 85 90 95  
 Ser Gly Glu Val Lys Thr Leu Asn Thr Ala Met Ala Gly Ala His Phe  
 100 105 110  
 Asp Phe Met Phe Asp Val Val Asn Phe Tyr Ala Lys Tyr Asn Pro Lys  
 115 120 125  
 Arg Val Phe His Leu Ile Pro Trp Phe Gly Val Gly Tyr Gly Phe Lys  
 130 135 140  
 Tyr Tyr Asn Asp Phe Ala Asp Leu Ala Asp Met Ile Gln Phe Asn Glu  
 145 150 155 160  
 Pro Phe Arg His Ser Ala Thr Ala Asn Ala Gly Leu Met Met Ser Phe  
 165 170 175  
 Arg Leu Ala Lys Arg Leu Asp Leu Val Leu Glu Gly Gln Ala Ile Tyr  
 180 185 190  
 Ser Asn Leu Asn Ile Val Lys Gln Glu Ile Asp Tyr Lys Ala Pro Ile  
 195 200 205  
 Met Pro Tyr Ser Asn Ile Tyr Asn Gly Leu Thr Gly Val Val Thr Ala  
 210 215 220  
 Gly Leu Asn Phe Asn Leu Gly Arg Val Ala Trp Glu Ser Val Thr Pro  
 225 230 235 240  
 Met Asp Met Asp Leu Ile Asn Asp Leu Asn Gly Gln Ile Asn Arg Leu  
 245 250 255  
 Arg Ser Glu Asn Thr Glu Leu Arg Lys Arg Pro Val Ser Cys Pro Glu  
 260 265 270

Cys Pro Glu Val Thr Ala Glu Thr Glu Val Val Thr Glu Asn Val Leu  
 275 280 285  
 Gly Asp Lys Ala Ile Val Phe Lys Phe Asn Ser Ala Thr Ile Asp Lys  
 290 295 300  
 Asp Gln His Ile Val Leu Gln Asp Ile Ala Asp Phe Val Lys Asp Gly  
 305 310 315 320  
 Asn Lys Ala Ile Val Val Ile Gly Phe Ala Asp Thr Thr Gly Asp Ile  
 325 330 335  
 Asn Tyr Asn Met His Leu Ser Glu Arg Arg Ala Lys Ala Val Ala Glu  
 340 345 350  
 Ala Leu Val Asn Lys Phe Gly Val Ser Ser Asp Met Ile Ser Val Glu  
 355 360 365  
 Trp Gln Gly Glu Thr Glu Gln Phe Asn Pro Arg Ala Trp Asn  
 370 375 380

<210> 123

<211> 375

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. gulae B69  
OprF polypeptide sequence

<400> 123

Thr Phe Val Gly Ala Ile Ala Leu Asn Ala Ser Ala Gln Glu Asn Thr  
 1 5 10 15  
 Val Pro Ala Thr Gly Gln Leu Pro Ala Lys Asn Val Ala Phe Ala Arg  
 20 25 30  
 Asn Lys Ala Gly Gly Asn Trp Phe Val Thr Leu Gln Gly Gly Val Ala  
 35 40 45  
 Ala Gln Phe Leu Asn Asp Asn Asn Asn Lys Asp Leu Val Asp Arg Leu  
 50 55 60  
 Gly Ala Thr Gly Ser Ile Ser Val Gly Lys Tyr His Asn Pro Phe Phe  
 65 70 75 80

Ala Thr Arg Leu Gln Ile Asn Gly Gly Gln Ala His Thr Phe Leu Gly	85	90	95
Lys Asn Ala Glu Gln Glu Ile Asn Thr Asn Phe Gly Ala Ala His Phe	100	105	110
Asp Phe Met Phe Asp Val Val Asn Tyr Phe Ala Pro Tyr Arg Glu Asn	115	120	125
Arg Phe Phe His Leu Ile Pro Trp Val Gly Val Gly Tyr Gln His Lys	130	135	140
Phe Ile Gly Ser Glu Trp Ser Lys Asp Asn Val Glu Ser Leu Thr Ala	145	150	155
Asn Met Gly Val Met Met Ala Phe Arg Leu Gly Lys Arg Val Asp Phe	165	170	175
Val Ile Glu Ala Gln Ala Ala His Ser Asn Leu Asn Leu Ser Arg Ala	180	185	190
Phe Asn Ala Lys Lys Thr Pro Ile Phe His Asp Gln Glu Gly Arg Tyr	195	200	205
Tyr Asn Gly Phe Gln Gly Met Ala Thr Ala Gly Leu Asn Phe Arg Leu	210	215	220
Gly Ala Val Gly Phe Asn Ala Ile Glu Pro Met Asp Tyr Ala Leu Ile	225	230	235
Asn Asp Leu Asn Gly Gln Ile Asn Arg Leu Arg Arg Glu Val Glu Glu	245	250	255
Leu Ser Lys Arg Pro Val Ser Cys Pro Glu Cys Pro Asp Val Thr Pro	260	265	270
Val Thr Lys Thr Glu Asn Lys Leu Thr Glu Lys Ala Val Leu Phe Arg	275	280	285
Phe Asp Ser Tyr Val Val Asp Lys Asp Gln Leu Ile Asn Leu Tyr Asp	290	295	300
Val Ala Gln Phe Val Lys Glu Thr Asn Glu Pro Ile Thr Val Val Gly	305	310	315
Tyr Ala Asp Pro Thr Gly Ser Thr Gln Tyr Asn Glu Arg Leu Ser Glu	325	330	335

Arg Arg Ala Lys Ala Val Val Asp Val Leu Thr Gly Lys Tyr Gly Val  
 340 345 350

Pro Ser Glu Leu Ile Ser Val Glu Trp Lys Gly Asp Ser Thr Gln Pro  
 355 360 365

Phe Asn Lys Lys Ala Trp Asn  
 370 375

<210> 124

<211> 382

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P.  
 circumdentaria B97 OprF polypeptide sequence

<400> 124

Ser Val Met Gly Ala Thr Ala Leu Thr Val Ser Ala Gln Gln Pro Thr  
 1 5 10 15

Thr Pro Glu Thr Gln Thr Leu Pro Ala His Lys Thr Ala Phe Asp Arg  
 20 25 30

Ser Ala Gly His Trp Phe Leu Thr Leu Gln Gly Gly Val Ser Ala Gln  
 35 40 45

Phe Leu Glu Glu Asn Glu Ser Gln Glu Ile Leu Asn Arg Leu His Val  
 50 55 60

Met Pro Thr Ile Ser Leu Gly Lys Trp His Asn Pro Tyr Phe Ala Thr  
 65 70 75 80

Arg Leu Gln Val Phe Gly Gly Pro Thr Pro Thr Phe Tyr Lys Asn Ala  
 85 90 95

Ala Gly Lys Val Met Lys Glu Asn Ala Ala Met Ala Gly Ala His Phe  
 100 105 110

Asp Phe Met Phe Asp Val Val Asn Tyr Phe Gly Lys Tyr Asn Pro Lys  
 115 120 125

Arg Val Phe His Leu Val Pro Trp Phe Gly Val Gly Tyr Gly Phe Lys  
 130 135 140

Tyr His Asn Asp Phe Ala Glu Met Ser Asp Ile Ile Lys Phe Asn Glu

145	150	155	160
Pro Tyr Arg His Ser Ala Thr Ala Asn Ala Gly Leu Met Met Ser Phe	165	170	175
Arg Leu Ala Lys Arg Leu Asp Leu Val Leu Glu Gly Gln Ala Ile Tyr	180	185	190
Ser Asn Leu Asn Ile Val Lys Gln Glu Ile Asp Tyr Lys Ala Pro Ser	195	200	205
Thr Pro Tyr Ser Pro Asn Tyr Asn Gly Leu Leu Gly Val Val Thr Ala	210	215	220
Gly Leu Asn Phe Asn Leu Gly Arg Val Ala Trp Glu Thr Val Thr Pro	225	230	235
Met Asp Met Asp Leu Ile Asn Asp Leu Asn Gly Gln Ile Asn Arg Leu	245	250	255
Arg Ser Glu Asn Thr Glu Leu Arg Lys Arg Pro Val Ser Cys Pro Glu	260	265	270
Cys Pro Glu Val Ser Lys Glu Thr Thr Val Val Thr Glu Asn Val Leu	275	280	285
Gly Asp Lys Ala Ile Val Phe Lys Phe Asn Ser Ala Thr Ile Ser Lys	290	295	300
Asp Gln His Ile Val Leu Gln Asp Ile Ala Asp Phe Val Lys Asn Gly	305	310	315
Asn Lys Gly Val Ala Val Ile Gly Phe Ala Asp Val Thr Gly Asp Ala	325	330	335
Asn Tyr Asn Met Gln Leu Ser Glu Arg Arg Ala Lys Ala Val Ala Glu	340	345	350
Ala Leu Val Asn Gln Phe Gly Val Pro Ser Asp Met Ile Ser Val Glu	355	360	365
Trp Gln Gly Glu Thr Glu Leu Phe Glu Ala Arg Ala Trp Asn	370	375	380

&lt;210&gt; 125

&lt;211&gt; 316

&lt;212&gt; PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P.  
cangingivalis B98 OprF polypeptide sequence

<400> 125

Gly Gly Val Ser Ala Gln Phe Leu Glu Glu Asn Glu Ser Gln Glu Ile  
1 5 10 15

Leu Asn Arg Leu His Val Met Pro Thr Ile Ser Leu Gly Lys Trp His  
20 25 30

Asn Pro Tyr Phe Ala Thr Arg Leu Gln Val Phe Gly Gly Pro Thr Pro  
35 40 45

Thr Phe Tyr Lys Asn Ala Ala Gly Lys Val Met Lys Glu Asn Ala Ala  
50 55 60

Met Ala Gly Ala His Phe Asp Phe Met Phe Asp Val Val Asn Tyr Phe  
65 70 75 80

Gly Lys Tyr Asn Pro Lys Arg Val Phe His Leu Val Pro Trp Phe Gly  
85 90 95

Val Gly Tyr Gly Phe Lys Tyr His Asn Asp Phe Ala Glu Met Ser Asp  
100 105 110

Ile Ile Lys Phe Asn Glu Pro Tyr Arg His Ser Ala Thr Ala Asn Ala  
115 120 125

Gly Leu Met Met Ser Phe Arg Leu Ala Lys Arg Leu Asp Leu Val Leu  
130 135 140

Glu Gly Gln Ala Ile Tyr Ser Asn Leu Asn Ile Val Lys Gln Glu Ile  
145 150 155 160

Asp Tyr Lys Ala Pro Ser Thr Pro Tyr Ser Pro Asn Tyr Asn Gly Leu  
165 170 175

Leu Gly Val Val Thr Ala Gly Leu Asn Phe Asn Leu Gly Arg Val Ala  
180 185 190

Trp Glu Thr Val Thr Pro Met Asp Met Asp Leu Ile Asn Asp Leu Asn  
195 200 205

Gly Gln Ile Asn Arg Leu Arg Ser Glu Asn Thr Glu Leu Arg Lys Arg  
210 215 220

Pro Val Ser Cys Pro Glu Cys Pro Glu Val Ser Lys Glu Thr Thr Val  
 225 230 235 240

Val Thr Glu Asn Val Leu Gly Asp Lys Ala Ile Val Phe Lys Phe Asn  
 245 250 255

Ser Ala Thr Ile Ser Lys Asp Gln His Ile Val Leu Gln Asp Ile Ala  
 260 265 270

Asp Phe Val Lys Asn Gly Asn Lys Gly Val Ala Val Ile Gly Phe Ala  
 275 280 285

Asp Val Thr Gly Asp Ala Asn Tyr Asn Met Gln Leu Ser Glu Arg Arg  
 290 295 300

Ala Lys Ala Val Ala Glu Ala Leu Val Asn Gln Phe  
 305 310 315

&lt;210&gt; 126

&lt;211&gt; 323

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:P. salivosa

B104 OprF polypeptide sequence

&lt;400&gt; 126

His Trp Phe Leu Thr Leu Gln Gly Gly Val Ser Ala Gln Phe Leu Glu  
 1 5 10 15

Glu Asn Glu Ser Gln Glu Ile Leu Asn Arg Leu His Val Met Pro Thr  
 20 25 30

Ile Ser Leu Gly Lys Trp His Asn Pro Tyr Phe Ala Thr Arg Leu Gln  
 35 40 45

Val Phe Gly Gly Pro Thr Pro Thr Phe Tyr Lys Asn Ala Ala Gly Lys  
 50 55 60

Val Met Lys Glu Asn Ala Ala Met Ala Gly Ala His Phe Asp Phe Met  
 65 70 75 80

Phe Asp Val Val Asn Tyr Phe Gly Lys Tyr Asn Pro Lys Arg Val Phe  
 85 90 95

His Leu Val Pro Trp Phe Gly Val Gly Tyr Gly Phe Lys Tyr His Asn  
 100 105 110  
 Asp Phe Ala Glu Met Ser Asp Ile Ile Lys Phe Asn Glu Pro Tyr Arg  
 115 120 125  
 His Ser Ala Thr Ala Asn Ala Gly Leu Met Met Ser Phe Arg Leu Ala  
 130 135 140  
 Lys Arg Leu Asp Leu Val Leu Glu Gly Gln Ala Ile Tyr Ser Asn Leu  
 145 150 155 160  
 Asn Ile Val Lys Gln Glu Ile Asp Tyr Lys Ala Pro Ser Thr Pro Tyr  
 165 170 175  
 Ser Pro Asn Tyr Asn Gly Leu Leu Gly Val Val Thr Ala Gly Leu Asn  
 180 185 190  
 Phe Asn Leu Gly Arg Val Ala Trp Glu Thr Ile Thr Pro Met Asp Met  
 195 200 205  
 Asp Leu Ile Asn Asp Leu Asn Gly Gln Ile Asn Arg Leu Arg Ser Glu  
 210 215 220  
 Asn Thr Glu Leu Arg Lys Arg Pro Val Ser Cys Pro Glu Cys Pro Glu  
 225 230 235 240  
 Val Ser Lys Glu Thr Thr Val Val Thr Glu Asn Val Leu Gly Asp Lys  
 245 250 255  
 Ala Ile Val Phe Lys Phe Asn Ser Ala Thr Ile Ser Lys Asp Gln His  
 260 265 270  
 Ile Val Leu Gln Asp Ile Ala Asp Phe Val Lys Asn Gly Asn Lys Gly  
 275 280 285  
 Val Ala Val Ile Gly Phe Ala Asp Val Thr Gly Asp Ala Asn Tyr Asn  
 290 295 300  
 Met Gln Leu Ser Glu Arg Arg Ala Lys Ala Val Ala Glu Ala Leu Val  
 305 310 315 320  
 Asn Gln Phe

&lt;210&gt; 127

&lt;211&gt; 349



&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. denticanis  
B106 OprF polypeptide sequence

&lt;400&gt; 127

Ala His Lys Thr Ala Phe Asp Arg Ser Ala Gly His Trp Phe Leu Thr  
1 5 10 15

Leu Gln Gly Gly Val Ser Ala Gln Phe Leu Glu Glu Asn Glu Ser Gln  
20 25 30

Glu Ile Leu Asn Arg Leu His Val Met Pro Thr Ile Ser Leu Gly Lys  
35 40 45

Trp His Asn Pro Tyr Phe Ala Thr Arg Leu Gln Val Phe Gly Gly Pro  
50 55 60

Thr Pro Thr Phe Tyr Lys Asn Ala Ala Gly Lys Val Met Lys Glu Asn  
65 70 75 80

Ala Ala Met Ala Gly Ala His Phe Asp Phe Met Phe Asp Val Val Asn  
85 90 95

Tyr Phe Gly Lys Tyr Asn Pro Lys Arg Val Phe His Leu Val Pro Trp  
100 105 110

Phe Gly Val Gly Tyr Gly Phe Lys Tyr His Asn Asp Phe Ala Glu Met  
115 120 125

Ser Asp Ile Ile Lys Phe Asn Glu Pro Tyr Arg His Ser Ala Thr Ala  
130 135 140

Asn Ala Gly Leu Met Met Ser Phe Arg Leu Ala Lys Arg Leu Asp Leu  
145 150 155 160

Val Leu Glu Gly Gln Ala Ile Tyr Ser Asn Leu Asn Ile Val Lys Gln  
165 170 175

Glu Ile Asp Tyr Lys Ala Pro Ser Thr Pro Tyr Ser Pro Asn Tyr Asn  
180 185 190

Gly Leu Leu Gly Val Val Thr Ala Gly Leu Asn Phe Asn Leu Gly Arg  
195 200 205

Val Ala Trp Glu Thr Val Thr Pro Met Asp Met Asp Leu Ile Asn Asp

210	215	220
Leu Asn Gly Gln Ile Asn Arg Leu Arg Ser Glu Asn Thr Glu Leu Arg		
225	230	235 240
Lys Arg Pro Val Ser Cys Pro Glu Cys Pro Glu Val Ser Lys Glu Thr		
245	250	255
Thr Val Val Thr Glu Asn Val Leu Gly Asp Lys Ala Ile Val Phe Lys		
260	265	270
Phe Asn Ser Ala Thr Ile Ser Lys Asp Gln His Ile Val Leu Gln Asp		
275	280	285
Ile Ala Asp Phe Val Lys Asn Gly Asn Lys Gly Val Ala Val Ile Gly		
290	295	300
Phe Ala Asp Val Thr Gly Asp Ala Asn Tyr Asn Met Gln Leu Ser Glu		
305	310	315 320
Arg Arg Ala Lys Ala Val Ala Glu Ala Leu Val Asn Gln Phe Gly Val		
325	330	335
Pro Ser Asp Met Ile Ser Val Glu Trp Gln Gly Glu Thr		
340	345	

&lt;210&gt; 128

&lt;211&gt; 395

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:P. endodontalis  
B114 OprF polypeptide sequence

&lt;400&gt; 128

Ser Ala Leu Gly Ala Leu Ala Leu Thr Ala Ser Ala Gln Gln Thr Thr
1 5 10 15
Lys Pro Ala Asn Ser Met Pro Ala Phe Lys Thr Ala Phe Glu Arg Ser
20 25 30
Gly Gly His Trp Phe Leu Thr Ile Gln Gly Gly Leu Ser Ala Gln Leu
35 40 45
Leu Gly Glu Asn Glu Lys Met Asp Phe Gly Lys Arg Leu Leu His Ala
50 55 60

Ala	Lys	Ala	Ser	Asp	Asn	Thr	Gln	Thr	Glu	Ala	Ser	Tyr	Leu	Arg	Ile	65	70	75	80
Met	Pro	Thr	Leu	Ser	Val	Gly	Lys	Trp	His	Asn	Pro	Tyr	Phe	Ala	Thr	85	90	95	
Arg	Val	Gln	Leu	Phe	Gly	Gly	Leu	Thr	Pro	Leu	Tyr	Asn	Thr	Glu	Gly	100	105	110	
Gly	Val	Asn	Val	His	Thr	Tyr	Asn	Thr	Ala	Thr	Ile	Gly	Ala	His	Tyr	115	120	125	
Asp	Phe	Met	Phe	Asp	Val	Val	Asn	Tyr	Phe	Ala	Lys	Tyr	Asn	Pro	Lys	130	135	140	
Arg	Phe	Phe	His	Val	Ile	Pro	Trp	Val	Gly	Leu	Gly	Tyr	Asn	Phe	Lys	145	150	155	160
Tyr	His	Asp	Val	Phe	Gly	Phe	Lys	Glu	Pro	Tyr	Arg	His	Ser	Val	Thr	165	170	175	
Gly	Asn	Ala	Gly	Met	Glu	Phe	Ala	Phe	Arg	Leu	Gly	Lys	Arg	Val	Asp	180	185	190	
Leu	Val	Leu	Glu	Ala	Gln	Val	Val	Tyr	Asn	Asn	Leu	Asn	Leu	Ile	Lys	195	200	205	
Gln	Glu	Val	Asp	Tyr	Asp	Val	Val	Thr	Thr	Pro	Tyr	Val	Pro	Ala	Asp	210	215	220	
Thr	Tyr	Ala	Gly	Leu	Met	Thr	Met	Phe	Thr	Ala	Gly	Leu	Asn	Phe	Asn	225	230	235	240
Leu	Gly	Lys	Val	Glu	Trp	Glu	Thr	Val	Glu	Pro	Met	Asp	Tyr	Gln	Leu	245	250	255	
Ile	Asn	Asp	Leu	Asn	Ser	Gln	Ile	Ser	Arg	Leu	Arg	Ser	Glu	Asn	Ala	260	265	270	
Glu	Leu	Ser	Lys	Arg	Pro	Ala	Phe	Cys	Pro	Glu	Cys	Pro	Glu	Val	Glu	275	280	285	
Glu	Val	Glu	Asp	Val	Val	Val	Asp	Gln	Tyr	Val	Leu	Thr	Asp	Lys	Ala	290	295	300	
Ile	Leu	Phe	Asp	Phe	Asp	Lys	Ser	Asn	Ile	Arg	Lys	Asp	Gln	Gln	Ala	305	310	315	320

Gln Leu Gly Met Ile Ala Glu Phe Val Lys Lys Tyr Asn Thr Pro Ile  
                           325                          330                          335

Val Val Val Gly Tyr Ala Asp Pro Thr Gly Lys Ser Lys Tyr Asn Met  
                           340                          345                          350

Glu Leu Ser Lys Arg Arg Ala Gln Ala Val Val Asn Glu Leu Thr Asn  
                           355                          360                          365

Arg His Gly Val Pro Ala Asp Leu Ile Thr Met Glu Trp Glu Gly Ala  
                           370                          375                          380

Thr Asn Lys Phe Thr Pro Pro Thr Ala Trp Asn  
                           385                          390                          395

<210> 129

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. gulae B43  
           FimA polypeptide fragment sequence #1

<400> 129

Ala Cys Asn Lys Asp Asn Glu Ala Glu Pro Val Val  
       1                          5                          10

<210> 130'

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. gulae B43  
           FimA polypeptide fragment sequence #2

<400> 130

Tyr Pro Val Leu Val Asn Phe Glu Ser Asn Asn Tyr Thr Tyr Thr Gly  
       1                          5                          10                          15

Asp Ala Val Glu Lys  
                           20

<210> 131  
 <211> 16  
 <212> PRT  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. guiae B43  
 FimA polypeptide fragment sequence #3

<400> 131

Thr Gly Pro Gly Thr Asn Asn Pro Glu Asn Pro Ile Thr Glu Ser Ala  
 1 5 10 15

<210> 132  
 <211> 14  
 <212> PRT  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. guiae B43  
 OprF polypeptide fragment sequence #1

<400> 132

Asn Asp Asn Asn Asn Lys Asp Phe Val Asp Arg Leu Gly Ala  
 1 5 10

<210> 133  
 <211> 29  
 <212> PRT  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. guiae B43  
 OprF polypeptide fragment sequence #2

<400> 133

Asp Leu Asn Gly Gln Ile Asn Arg Leu Arg Arg Glu Val Glu Glu Leu  
 1 5 10 15

Ser Lys Arg Pro Val Ser Cys Pro Glu Cys Pro Asp Val  
 20 25

<210> 134  
 <211> 21  
 <212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:P. guiae B43  
OprF polypeptide fragment sequence #3

<400> 134

Ala Asp Pro Thr Gly Asp Thr Gln Tyr Asn Glu Arg Leu Ser Glu Arg  
1 5 10 15

Arg Ala Lys Ala Val  
20

<210> 135

<211> 47

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:pBAD-HisA  
Amino-terminal polypeptide sequence

<400> 135

Met Gly Gly Ser His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp Arg  
20 25 30

Trp Gly Ser Glu Leu Glu Ile Cys Ser Gln Tyr His Met Gly Ile  
35 40 45

<210> 136

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:pBAD-TOPO  
Amino-terminal polypeptide sequence

<400> 136

Met Gly Ser Gly Ser Gly Asp Asp Asp Asp Lys Leu Ala Leu Met  
1 5 10 15

<210> 137

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:1 vector  
Amino-terminal polypeptide sequence

<400> 137

Met Gly Thr Thr Thr Thr Thr Thr Ser Leu His Met  
1 5 10

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(54) Title: VACCINE FOR PERIODONTAL DISEASE

(57) Abstract: The present invention relates to bacterial isolates identified by their 16S rRNA DNA, that cause periodontal disease in companion animals, polynucleotide sequences contained therein, polypeptides encoded by such polynucleotide sequences and vaccines comprising such bacteria, polynucleotides, or polypeptides. Also provided are methods for treating and preventing peri-odontal disease and kits for detecting and treating periodontal disease kits for detecting and preventing periodontal disease.



## INTERNATIONAL SEARCH REPORT

PCT/IB 02/05539

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N1/20 C12N15/31 C12N15/63 C07K14/195

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, EMBL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DOMINIQUE FOURNIER ET AL.: "Porphyromonas gulae sp. nov., an anaerobic Gram-negative coccobacillus from the gingival ulcer of various animal hosts" INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, vol. 51, no. 3, May 2001 (2001-05), pages 1179-1189, XP008015632 abstract page 1182, left-hand column, paragraph 2 -page 1183, right-hand column, paragraph 2; table 2	2,4-6
X	& DATABASE EMBL [Online] Entry AF285873, 29 August 2000 (2000-08-29) FOURNIER D. ET AL.: "Porphyromonas gulae strain chien 4.2 16S ribosomal RNA gene, partial sequence" the whole document -/--	1-7,12



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

7 April 2003

Date of mailing of the international search report

07. 07. 2003

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MONTERO LOPEZ B.

## INTERNATIONAL SEARCH REPORT

PCT/IB 02/05539

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>---  M.D. COLLINS ET AL.: "Phylogenetic analysis of members of the genus Porphyromonas and description of Porphyromonas cangingivalis sp. nov. and Porphyromonas cansulci sp. nov." INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, vol. 44, no. 1, January 1994 (1994-01), pages 674-679, XP008015640  abstract  page 674, right-hand column, paragraph 3  -page 675, left-hand column, paragraph 2  page 675, right-hand column, paragraph 2  -page 679, left-hand column, last paragraph</p>	2,4-6
X	<p>---  DATABASE EMBL [Online]  Entry AF414811,  1 October 2001 (2001-10-01)  KUHNERT P. ET AL.: "Porphyromonas gingivalis strain 2-PGI 16S ribosomal RNA gene, partial sequence"  Database accession no. AF414811  XP002237326  the whole document  &amp; PETER KUHNERT ET AL.: "Phylogenetic analysis of Prevotella nigrescens, Prevotella intermedia and Porphyromonas gingivalis clinical strains reveals a clear species clustering" INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, vol. 52, July 2002 (2002-07), pages 1391-1395, XP008015995</p>	1-7,12
X	<p>---  BRUCE J. PASTER ET AL.: "Phylogeny of Bacteroides, Prevotella, and Porphyromonas spp. and related bacteria" JOURNAL OF BACTERIOLOGY, vol. 176, no. 3, February 1994 (1994-02), pages 725-732, XP008015996  abstract  page 727, left-hand column, paragraph 2  -right-hand column, paragraph 2</p>	1-7,12
X	<p>&amp; DATABASE EMBL [Online]  Entry POYRR16SC, 20 May 1993 (1993-05-20)  PASTER B.J. ET AL.: "Porphyromonas gingivalis ATCC 33277 16S ribosomal RNA gene, complete sequence"  Database accession no. L16492  the whole document</p> <p>---  -/--</p>	1-7,12

## INTERNATIONAL SEARCH REPORT

PCT/IB 02/05539

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 6 444 799 B1 (BRUCE CARTER ROSS) 3 September 2002 (2002-09-03) column 1, line 15 -column 8, line 42 sequence listing SEQ ID NO:115 & DATABASE EMBL [Online] Entry AR226655, 21 December 2002 (2002-12-21) ROSS B.C.: "Sequence 115 from patent US 6444799" the whole document ---	1-7,12
A	US 5 710 039 A (MASATOMO HIRASAWA ET AL.) 20 January 1998 (1998-01-20) cited in the application column 1, line 36 - line 62 ---	1-6
A	BRUCE J. PASTER: "Bacterial diversity in human subgingival plaque" JOURNAL OF BACTERIOLOGY, vol. 183, no. 12, June 2001 (2001-06), pages 3770-3783, XP002237325 abstract page 3771, right-hand column, paragraph 2 - paragraph 4 page 3772, left-hand column, paragraph 5 -----	1-3

# INTERNATIONAL SEARCH REPORT

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## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

partially 1-7 and 12

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: partially 1-7 and 12

Porphyromonas gulae B43 16S RNA polynucleotide sequence of SEQ ID NO:86; bacteria comprising it

2. Claims: partially 1-7 and 12

Idem as subject 1 for respectively:

Porphyromonas cansulci B46 16S RNA of SEQ ID NO:87  
 Porphyromonas circumdentari B52 16S RNA of SEQ ID NO:88  
 Porphyromonas gulae B69 16S RNA of SEQ ID NO:89  
 Porphyromonas circumdentari B97 16S RNA of SEQ ID NO:90  
 Porphyromonas cangingivalis B98 16S RNA of SEQ ID NO:91  
 Porphyromonas salivosa B104 16S RNA of SEQ ID NO:92  
 Porphyromonas denticanis B106 16S RNA of SEQ ID NO:93  
 Porphyromonas endodontalis B114 16S RNA of SEQ ID NO:94

3. Claims: partially 8-15

Porphyromonas gulae B43 fimA polynucleotide sequence of SEQ ID NO:95; vector and host cell comprising it and use thereof for production of the encoded polypeptide; encoded polypeptide of sequence SEQ ID N:103

4. Claims: partially 8-15

Idem as subject 10 for respectively:

Porphyromonas circumdentari B52 fimA polynucleotide sequence of SEQ ID NO:96 and encoded polypeptide of sequence SEQ ID NO:104  
 Porphyromonas gulae B69 fimA polynucleotide sequence of SEQ ID NO:97 and encoded polypeptide of sequence SEQ ID NO:105  
 Porphyromonas circumdentari B57 fimA polynucleotide sequence of SEQ ID NO:98 and encoded polypeptide of sequence SEQ ID NO:106  
 Porphyromonas cangingivalis B98 fimA polynucleotide sequence of SEQ ID NO:99 and encoded polypeptide of sequence SEQ ID NO:107  
 Porphyromonas salivosa B104 fimA polynucleotide sequence of SEQ ID NO:100 and encoded polypeptide of sequence SEQ ID NO:108  
 Porphyromonas denticanis B106 fimA polynucleotide sequence of SEQ ID NO:101 and encoded polypeptide of sequence SEQ ID NO:109  
 Porphyromonas endodontalis B114 fimA polynucleotide sequence of SEQ ID NO:102 and encoded polypeptide of sequence SEQ ID NO:110  
 Porphyromonas gulae B43 oprF polynucleotide sequence of SEQ ID NO:111 and encoded polypeptide of sequence SEQ ID NO:120

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Porphyromonas cansulci B46 oprF polynucleotide sequence of SEQ ID NO:112 and encoded polypeptide of sequence SEQ ID NO:121

Porphyromonas circumdentari B52 oprF polynucleotide sequence of SEQ ID NO:113 and encoded polypeptide of sequence SEQ ID NO:122

Porphyromonas gulae B69 oprF polynucleotide sequence of SEQ ID NO:114 and encoded polypeptide of sequence SEQ ID NO:123

Porphyromonas circumdentari B97 oprF polynucleotide sequence of SEQ ID NO:115 and encoded polypeptide of sequence SEQ ID NO:124

Porphyromonas camgingivalis B98 oprF polynucleotide sequence of SEQ ID NO:116 and encoded polypeptide of sequence SEQ ID NO:125

Porphyromonas salivisa B104 oprF polynucleotide sequence of SEQ ID NO:117 and encoded polypeptide of sequence SEQ ID NO:126

Porphyromonas denticanis B106 oprF polynucleotide sequence of SEQ ID NO:118 and encoded polypeptide of sequence SEQ ID NO:127

Porphyromonas endodontalis B114 oprf polynucleotide sequence of SEQ ID NO:119 and encoded polypeptide of sequence SEQ ID NO:128

# INTERNATIONAL SEARCH REPORT

International Application No

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US 5710039	A	20-01-1998	NONE
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